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64 The gal operon of streptomyces.

57 A recombinant DNA molecule comprising the *Streptomyces gal* operon *galK* gene; *galE* gene; *galT* gene; P1 promoter; P2 promoter; P2 promoter expression unit; P1 promoter regulated region; or the entire *Streptomyces gal* operon is prepared.

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TITLETHE GAL OPERON OF
STREPTOMYCESCROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of Serial Number 834,706, filed February 28, 1986, which is pending.

BACKGROUND OF THE INVENTION

This invention relates to a recombinant DNA molecule comprising the Streptomyces gal operon.

Hodgson, J. Gen. Micro., 128, 2417-2430 (1982), report that Streptomyces coelicolor A3(2) has a glucose repression system which allows repression at the level of transcription of the arabinose uptake system, one of the glycerol uptake systems, and also repression of the galactose uptake system in wild type strains. There is no report in Hodgson of actual galactose metabolism by S. coelicolor A3(2).

Okeda et al. Mol. Gen. Genet., 196, 501-507 (1984), report that glucose kinase activity, 2-deoxyglucose-sensitivity, glucose utilization and glucose repression were all restored to S. coelicolor A3(2) glk (glucose kinase) mutants transformed by a 3.5 kb DNA fragment which contained the glk gene cloned from S. coelicolor into a phage vector.

1 Seno et al., Mol. Gen. Genet., 193, 119-128
(1984), report the glycerol (gyl) operon of Streptomyces
coelicolor, and state that such operon is substrate-
inducible and catabolite-repressible.

5 Debouck et al., Nuc. Acids. Res., 13(6), 1841-1853
(1985), report that the gal operon of E. coli consists of
three structurally contiguous genes which specify the
enzymes required for the metabolism of galactose, i.e.,
10 galE (uridine diphosphogalactose-4-epimerase), galT
(galactose-1-phosphate uridylyltransferase) and galK
(galactokinase); that such genes are expressed from a
polycistronic mRNA in the order E, T, K; that the
expression of the promoter distal gene of the operon,
15 galK, is known to be coupled translationally to the galT
gene immediately preceding it; that such translational
coupling results from a structural overlap between the end
of the galT coding sequence and the ribosome binding
region of galK; and that the translational coupling of
20 galT and galK ensures the coordinate expression of these
genes during the metabolism of galactose.

SUMMARY OF THE INVENTION

This invention relates to a recombinant DNA
molecule comprising a Streptomyces gal operon galK gene;
galE gene; galT gene; P2 promoter expression unit, or P2
25 promoter or any functional derivative thereof as well as a
recombinant DNA molecule comprising a Streptomyces gal
operon P1 promoter, P1 promoter regulated region or the
entire gal operon or any regulatable and functional
derivative thereof.

30 This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon or any
regulatable and functional derivative thereof and a
functional DNA molecule operatively linked to such operon;
a recombinant DNA vector comprising and such DNA molecule,
35 and, optionally, additionally comprising a replicon; a
method of preparing a host cell transformed with such

1 vector; the transformed host prepared by such method; a
method of expressing such functional DNA sequence which
comprises cultivating such transformed host under suitable
5 conditions such that the functional DNA sequence is
expressed; and to a method of regulating the expression of
such functional DNA sequence which comprises cultivating
such transformed host under conditions which regulate such
expression.

10 This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon P2
promoter expression unit or any functional derivative
thereof and a functional DNA molecule operatively linked
to such unit; a recombinant DNA vector comprising such DNA
15 molecule, and, optionally, additionally comprising a
replicon; a method of preparing a host cell transformed
with such vector; the transformed host prepared by such
method; and to a method of expressing such functional DNA
sequence which comprises cultivating such transformed host
under suitable conditions such that the functional DNA
20 sequence is expressed.

This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon P1
promoter regulated region or any regulatable and
functional derivative thereof and a functional DNA
25 molecule operatively linked to such region; a recombinant
DNA vector comprising such DNA molecule, and, optionally,
additionally comprising a replicon; a method of preparing
a host cell transformed with such vector; the transformed
host prepared by such method; a method of expressing such
30 functional DNA sequence which comprises cultivating such
transformed host under suitable conditions such that the
functional DNA sequence is expressed; and to a method of
regulating the expression of such functional DNA sequence
which comprises cultivating such transformed host under
35 conditions which regulate such expression.

This invention also relates to a recombinant DNA

1 molecule comprising the Streptomyces gal operon P1
promoter or any regulatable and functional derivative
thereof and a foreign functional DNA molecule operatively
linked to such region; a recombinant DNA vector comprising
5 such DNA molecule, and, optionally, additionally
comprising a replicon; a method of preparing a host cell
transformed with such vector; the transformed host
prepared by such method; a method of expressing such
functional DNA sequence which comprises cultivating such
10 transformed host under suitable conditions such that the
functional DNA sequence is expressed; and to a method of
regulating the expression of such functional DNA sequence
which comprises cultivating such transformed host under
conditions which regulate such expression.

15 This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon P2
promoter or any functional derivative thereof and a
foreign functional DNA molecule operatively linked to such
region; a recombinant DNA vector comprising such DNA
20 molecule, and, optionally, additionally comprising a
replicon; a method of preparing a host cell transformed
with such vector; the transformed host prepared by such
method; and to a method of expressing such functional DNA
sequence which comprises cultivating such transformed host
25 under suitable conditions such that the functional DNA
sequence is expressed.

This invention also relates to a method of
enabling a non-galactose utilizing host microorganism or
cell to utilize galactose which comprises transforming
30 such host with a recombinant DNA molecule comprising a
Streptomyces gal operon or any portion of the Streptomyces
gal operon, or any functional derivative thereof, which is
adequate to enable such transformed host to utilize
galactose, This invention also relates to the recombinant
35 DNA vector employed in such method and to the host
prepared by such method.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents a restriction endonuclease map of the Streptomyces lividans 1326 galactose (gal) operon and indicates approximate locations for structural genes and promoters within the operon.

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Figure 2 represents a restriction endonuclease map of plasmid pK21.

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Figure 3 represents a comparison of the restriction endonuclease maps of the S. lividans gal operon and a restriction fragment containing the S. coelicolor galK gene.

DETAILED DESCRIPTION OF THE INVENTION

It has now been discovered that the Streptomyces genome contains a operon for the metabolism of galactose (i.e., a gal operon) which comprises three structural genes (galT, galE and galK) and two promoters (P1 and P2). The galT gene product is known as galactose-1-phosphate uridylyltransferase (transferase), the galE gene product is known as uridine diphosphogalactose-4-epimerase (epimerase), and the galK gene product is known as galactose-1-kinase (galactokinase). The function of the gene products of galT, galE and galK in galactose metabolism in Streptomyces is explained by the following diagram:

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1. galactose + ATP galactokinase
galactose-1-phosphate + ADP
2. galactose-1-phosphate + UDP-glucose transferase
UDP-galactose + glucose-1-phosphate
3. UDP-galactose epimerase UDP-glucose

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By the term "promoter" is meant any region upstream of a structural gene which permits binding of RNA polymerase and transcription to occur.

1 By the term "structural gene" is meant a coding
sequence for a polypeptide which serves to be the template
for the synthesis of mRNA.

5 By the term "operon" is meant a group of closely
linked genes responsible for the synthesis of one or a
group of enzymes which are functionally related as members
of one enzyme system. An operon comprises an operator
gene, a number of structural genes (equivalent to the
number of enzymes in the system) and a regulator gene. By
10 "operator" or "operator gene" is meant a DNA sequence
which controls the biosynthesis of the contiguous
structural gene(s) within an operon. By "regulator gene"
is meant a gene which controls the operator gene in an
operon through the production of a repressor which can be
15 either active (enzyme induction) or inactive (enzyme
repression). The transcription of the structural gene(s)
in an operon is switched on or off by the operator gene
which is itself controlled in one or more of three ways:
1) in inducible enzyme systems, the operator is switched
20 off by a repressor produced by the regulator gene and
which can be inactivated by some metabolite or signal
substance (an inducer) coming from elsewhere in the cell
or outside the cell, so that the presence of the inducer
results in the operon becoming active; or 2) in repressed
25 enzyme systems, the operator is switched off by a
repressor-corepressor complex which is a combination of an
inactive repressor produced by the regulator gene with a
corepressor from elsewhere, so that the presence of the
corepressor renders the operon inactive; or 3) in
30 activated gene systems, the promoter is switched on by an
activator produced by a regulator gene which can be
activated by some metabolic or signal substance.

The Streptomyces gal operon is naturally present
in the Streptomyces genome.

35 By the term "Streptomyces gal operon" is meant
that region of the Streptomyces genome which comprises the

1 P1 promoter, P2 promoter, galT, galE and galK structural
genes and any other regulatory regions required for
transcription and translation of such structural genes.

5 By the term "regulatory region" is meant a DNA
sequence, such as a promoter or operator, which regulates
transcription of a structural gene.

The following model is suggested for gene
expression within the Streptomyces gal operon. The P1
10 promoter is a galactose inducible promoter (i.e., it is
induced in the presence of galactose and repressed in the
presence of glucose). According to S1 data, the P2
promoter is constitutive, i.e., it is "turned on"
regardless of the presence or absence of galactose or any
other carbon source.

15 A cosmid library was constructed for Streptomyces
lividans 1326 DNA by using cosmid pJW357 (which encodes
the ability to replicate in both Streptomyces and E.
coli). This library was then transfected into E. coli K21
20 which is a derivative of the E. coli strain MM294 which
contained a bacteriophage P1 transduced galactokinase
(galK) mutation. Transfected cells were plated under
media conditions which select for both the presence of the
cosmid and the presence of an active galK gene. Weakly
positive colonies were isolated and the cosmid DNA derived
25 from these colonies was transformed into the K21 strain.
These transformations yielded two cosmids which
consistently produced positive growth with galactose as
the only carbon source. These galK⁺ cosmids were then
transformed into a Streptomyces host (i.e., Streptomyces
30 lividans 1326-12K) which had been isolated by the
inventors of the subject invention as unable to grow on
medium in which galactose was the only carbon source by
using 2-deoxy-galactose selection [see, Brawner et al.,
Gene, 40 191 (1985), in press]. Under conditions which
35 differentiate strains able and unable to produce

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galactokinase, only one of the cosmids caused the Streptomyces lividans 1326-12K host to become galK⁺.

5 Further studies have demonstrated that this cosmid encodes a gene with galactokinase activity. Additional studies, including DNA sequence analysis and protein studies demonstrate that this Streptomyces gene shares homology with the E. coli and yeast galactokinase genes. Regulation studies indicate that the cosmid encoded
10 galactokinase gene regulated in the same manner as the chromosome encoded gene.

A. S. lividans gal operon was originally isolated from a ca. 9 kilobase (Kb) region of Streptomyces lividans 1326. The ca. 9 Kb region of Streptomyces lividans 1326
15 containing the Streptomyces gal operon has been mapped substantially as follows in Table A. By "substantially" is meant (i) that the relative positions of the restriction sites are approximate, (ii) that one or more restriction sites can be lost or gained by mutations not
20 otherwise significantly affecting the operon, and (iii) that additional sites for the indicated enzymes and, especially for enzymes not tested, may exist. The restriction enzymes used herein are commercially available. All are described by Roberts, Nuc. Acids.
25 Res., 10(5): p117 (1982).

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TABLE A

	Map Position	Restriction Enzyme	Location (kb)
5	1	<u>HindIII</u>	-.40
	1a	<u>NruI</u>	0
	2	<u>BglII</u>	.75
	3	<u>EcoRI</u>	1.05
	4	<u>PvuII</u>	1.15
10	5	<u>MluI</u>	2.30
	6	<u>PvuII</u>	2.80
	7	<u>EcoRI</u>	4.00
	8	<u>PvuII</u>	4.10
	8a	<u>SacI</u>	4.25
15	9	<u>PvuII</u>	5.00
	10	<u>XhoI</u>	5.50
	11	<u>BamHI</u>	5.80
	12	<u>BamHI</u>	6.50
	13	<u>MluI</u>	6.90
20	13a	<u>PvuII</u>	7.20
	14	<u>MluI</u>	7.80
	15	<u>BamHI</u>	8.00
	16	<u>SphI</u>	8.30

25 Figure 1 represents a restriction endonuclease map of the Streptomyces lividans 1326 gal operon and indicates locations for structural genes (galT, galE and galK) and promoters (P1 and P2) comprised within the operon.

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Referring to Table A and Figure 1, the location of the promoters and structural genes of the Streptomyces lividans 1326 gal operon are mapped substantially as follows in Table B:

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TABLE B

		<u>Location (Kb)</u>
10	P1 transcription start site	.10
	<u>galT</u> translation initiation codon	.15
	P2 transcription start site	1.25
	<u>galE</u> translation initiation codon	1.50
	<u>galK</u> translation initiation codon	2.40
15	3' end of <u>galK</u> message	3.60

Microorganisms of the genus Streptomyces have historically been used as a source of antibiotics for the pharmaceutical industry. Consequently, the technical skills necessary to scale-up the production of biological products using Streptomyces as the vehicle for the production of such products are presently available. However, before Streptomyces can be used as a vehicle for the production of bioactive molecules using the new recombinant DNA technologies, there is a need to define regulatory elements in Streptomyces analogous to those which have proved useful in E. coli. These regulatory elements include ribosomal binding sites and regulated transcriptional elements.

The existence of a galE, galT or galK gene or gene product or gal operon in Streptomyces has not been previously reported. The instant invention, i.e., the cloning of the Streptomyces gal operon, enables construction of regulatable expression/cloning vectors in Streptomyces, other actinomycetes, and other host organisms. Furthermore, the instant invention led to the discovery that the Streptomyces gal operon is

1 polycistronic. perhaps the most important feature of the
cloning of the Streptomyces gal operon is the observation
that there are sequences essential for regulation of the
Streptomyces galK gene. Direct analogy to the initial use
5 of the lac promoter from E. coli as an expression system
can be made. In fact, Brosius et al., Proc. Natl. Acad.
Sci. USA, 81, 6929-6933 (1984), utilized the regulatory
elements of the E. coli lac promoter to regulate the
exceptionally strong E. coli ribosomal promoters. Because
10 it is likely that the Streptomyces gal operon ribosomal
promoters are also exceptionally strong, such promoters
enable the construction of regulatable expression vectors
which will be very useful in Streptomyces, other
actinomycetes, and other host organisms. The instant
15 invention also enabled the unexpected discovery that the
2-deoxygalactose selection which has been used in E. coli
to select for galK mutants also operates in Streptomyces
to select for galK mutants [see, Brawner et al., Gene 40,
191 (1985), in press]. This observation, combined with
20 the ability to clone the Streptomyces galK gene and the
promoter and regulatory regions required for its
transcription and translation on a cosmid, as described
herein, allows the direct insertion of any structural gene
into the chromosomally located galK gene of Streptomyces
25 by homologous recombination. This manipulation will allow
molecular biologists to stably insert DNA fragments of
interest into the Streptomyces chromosome. Such an
approach will allow researchers to tag or mark a
Streptomyces strain of interest or to insert expression
30 cassettes into the organism without the need of
maintaining an antibiotic selection such as that presently
required by most Streptomyces expression vectors.

This invention relates to a recombinant DNA
molecule comprising the Streptomyces gal operon or any
35 regulatable and functional derivative thereof.

1 By "regulatable and functional derivative" is meant any
derivative of the Streptomyces gal operon which functions
in substantially the same way as the naturally occurring
Streptomyces gal operon in terms of regulatable production
5 of the galT, galE and galK gene products. Such
derivatives include partial sequences of the gal operon,
as well as derivatives produced by modification of the gal
operon coding sequence. Techniques for modifying the gal
operon which are known in the art include, for example,
10 treatment with chemical mutagens, irradiation or direct
genetic engineering, such as by inserting, deleting or
substituting nucleic acids by the use of enzymes or
recombination techniques. The naturally occurring
Streptomyces gal operon can be isolated from any galactose
15 utilizing Streptomyces strain by employing the techniques
described herein. Numerous strains of various
Streptomyces species are publicly available from many
sources. For example, the American Type Culture
Collection, Rockville, Maryland, U.S.A. has approximately
20 400 different species of Streptomyces available to the
public. The ability of a particular strain of
Streptomyces to utilize galactose can be readily
determined by conventional techniques, such as by growing
such strain on a medium containing galactose as the sole
25 carbon source. The preferred Streptomyces species from
which to isolate a gal operon include S. lividans, S.
coelicolor, S. azureus and S. albus, S. carzinostaticus,
S. antifibrinolyticus and S. longisporus. S. lividans is
most preferred. The Streptomyces gal operon, and smaller
30 portions thereof, is useful as a nucleic acid probe to
obtain homologous sequences from other cells and
organisms. The Streptomyces gal operon is also useful as
a selection marker in an appropriate host mutant, and for
providing regulatory elements. By "appropriate host
35 mutant" is meant a host which does not utilize galactose

1 because it (a) does not contain a gal operon or (b)
contains a nonfunctional gal operon, or (c) contains a
defect within a homologous structural gene or regulatory
region comprised by the Streptomyces gal operon such as a
5 defective P1 promoter, P2 promoter, galT gene, galK gene
and/or galE gene. Thus, a recombinant DNA molecule
(comprising the Streptomyces gal operon and a foreign
functional DNA sequence operatively linked thereto), which
can be prepared by conventional techniques, can be
10 transformed into an appropriate host mutant by
conventional techniques for incorporation into the host
genome by homologous recombination to enable regulatable
expression of the foreign functional DNA sequence without
the need of maintaining an expensive antibiotic
15 selection. Such operon may therefore also be incorporated
on recombinant DNA expression vectors for regulatable
expression of a foreign functional DNA sequence
operatively linked to such operon in an appropriate host
mutant transformed with such vector without the need of
20 maintaining an expensive antibiotic selection. Such
operon is also useful for transforming those cells,
viruses and microorganisms, such as strains of
Streptomyces, other actinomycetes, and other prokaryotic
organisms, such as gal⁻ E. coli strains, which do not
25 utilize galactose into galactose utilizing strains. Such
transformation may have pleiotrophic effects on the
transformed host. By the term "functional DNA sequence"
is meant any discrete region of DNA derived directly or
indirectly from Streptomyces or any other source which
30 functions in a host organism transformed therewith as a
gene expression unit, structural gene, promoter or a
regulatory region. Preferred functional DNA sequences
include those coding for polypeptides of pharmaceutical
importance, such as, but not limited to, insulin, growth
35 hormone, tissue plasminogen activator, alpha -1-anti-
trypsin or antigens used in vaccine production. By the

1 term "foreign functional DNA sequence" is meant a functional DNA sequence not derived from the Streptomyces gal operon coding region.

5 This invention also relates to a recombinant DNA molecule comprising the Streptomyces gal operon P2 promoter expression unit or any functional derivative thereof. By the term "P2 promoter expression unit" is meant that region of the Streptomyces gal operon comprising the Streptomyces gal operon P2 promoter, galE and galK structural genes and any other regulatory regions
10 required for transcription and translation of such structural genes. By "functional derivative" is meant any derivative of the Streptomyces gal operon P2 promoter expression unit which functions in substantially the same way as the naturally occurring region in terms of
15 production of the Streptomyces gal operon galE and galK gene products. Such derivatives include partial sequences of the Streptomyces gal operon P2 promoter expression unit, as well as derivatives produced by modification of
20 the Streptomyces gal operon P2 promoter expression unit coding sequence. Techniques for effecting such modification are known in the art, and some have been outlined above. The naturally occurring Streptomyces gal operon P2 promoter expression unit can be isolated from
25 the naturally occurring Streptomyces gal operon by conventional techniques. The Streptomyces gal operon P2 expression unit is useful as a selection marker in an appropriate host mutant and for providing regulatory elements. By "appropriate host mutant" is meant a host
30 which does not utilize galactose because it contains a defect within a homologous structural gene or regulatory region comprised by the Streptomyces P2 promoter expression unit such as a defective P2 promoter, galE gene and/or galK gene. Thus, a recombinant DNA molecule
35 (comprising the Streptomyces gal operon P2 promoter expression unit and a foreign functional DNA sequence

1 operatively linked thereto), which can be prepared by
conventional techniques, can be transformed into an
appropriate host mutant by conventional techniques for
incorporation into the host genome by homologous
5 recombination to enable constitutive expression of the
foreign functional DNA sequence without the need of
maintaining an expensive antibiotic selection. Such
expression unit may also be incorporated on recombinant
DNA expression vectors for constitutive expression of
10 foreign functional DNA sequences. The Streptomyces gal
operon P2 promoter expression unit is also useful for
complementation of an appropriate host mutant which can
then be used for constitutive expression of a foreign
functional DNA sequence operatively linked to such
15 expression unit in an appropriate host mutant transformed
with such vector without the need of maintaining an
expensive antibiotic selection.

This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon P1
20 promoter regulated region or any regulatable and
functional derivative thereof. By the term "P1 promoter
regulated region" is meant that region of the Streptomyces
gal operon comprising the Streptomyces gal operon P1
promoter, galT, galE and galK structural genes and any
25 other regulatory regions required for transcription and
translation of such structural genes. By "regulatable and
functional derivative" is meant any derivative of the
Streptomyces gal operon P1 promoter regulated region which
functions in substantially the same way as the naturally
30 occurring region in terms of regulatable production of the
Streptomyces gal operon galT, galE and galK gene
products. Such derivatives include partial sequences of
the Streptomyces gal operon P1 promoter regulated region,
as well as derivatives produced by modification of the
35 Streptomyces gal operon P1 promoter regulated region
coding sequence. Techniques for effecting such

1 modification are known in the art, and some have been
outlined above. The naturally occurring Streptomyces gal
operon P1 promoter regulated region can be isolated from
the naturally occurring Streptomyces gal operon by
5 conventional techniques, such as by excising the P2
promoter from the naturally occurring Streptomyces gal
operon or inactivating the P2 promoter by a point mutation
or by inserting a foreign DNA sequence within the
promoter. The Streptomyces gal operon P1 promoter
10 regulated region is useful for the utilities outlined
above for the Streptomyces gal operon.

This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon P2
promoter or any functional derivative thereof. By
"functional derivative" is meant any derivative of the
15 Streptomyces gal operon P2 promoter which functions in
substantially the same way as the naturally occurring P2
promoter in terms of enabling the binding of RNA
polymerase thereto and transcription of a functional DNA
sequence operatively linked to such promoter. Such
20 derivatives include partial sequences of the Streptomyces
gal operon P2 promoter, as well as derivatives produced by
modification of the gal operon P2 promoter coding
sequence. Techniques for effecting such modification are
known in the art, and some have been outlined above. The
25 naturally occurring Streptomyces gal operon P2 promoter
can be isolated from the naturally occurring Streptomyces
gal operon by conventional techniques. A recombinant DNA
molecule (comprising the Streptomyces gal operon P2
promoter and a foreign functional DNA sequence operatively
30 linked thereto), which can be prepared by conventional
techniques, can be transformed into an appropriate host
mutant by conventional techniques for incorporation into
the host genome by homologous recombination to enable
constitutive expression of the foreign functional DNA
35 sequence. The Streptomyces gal operon P2 promoter is also

1 useful for incorporation into recombinant DNA expression
vectors for constitutive expression of a foreign
functional DNA sequence operatively linked thereto in
viruses and eukaryotic or prokaryotic cells or organisms,
5 especially in Streptomyces or other actinomycetes,
transformed with such vector.

This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon P1
promoter or any regulatable and functional derivative
10 thereof. By "regulatable and functional derivative" is
meant any derivative of the Streptomyces gal operon P1
promoter which functions in substantially the same way as
the naturally occurring P1 promoter in terms of enabling
the binding of RNA polymerase thereto and regulating the
15 transcription of a functional DNA sequence operatively
linked to such promoter. Such derivatives include partial
sequences of the Streptomyces gal operon P1 promoter, as
well as derivatives produced by modification of the gal
operon P1 promoter coding sequence. Techniques for
20 effecting such modification are known in the art, and some
have been outlined above. The naturally occurring
Streptomyces gal operon P1 promoter can be isolated from
the naturally occurring Streptomyces gal operon by
conventional techniques. A recombinant DNA molecule
25 (comprising the Streptomyces gal operon P1 promoter and a
foreign functional DNA sequence operatively linked
thereto), which can be prepared by conventional
techniques, can be transformed into an appropriate host
mutant by conventional techniques for incorporation into
30 the host genome by homologous recombination to enable
regulatable expression of the foreign functional DNA
sequence. The Streptomyces gal operon P1 promoter is also
useful for incorporation into recombinant DNA expression
vectors for regulatable expression of a foreign functional
35 DNA sequence operatively linked thereto in viruses and
eukaryotic or prokaryotic cells or organisms, especially

1 Streptomyces or other actinomycetes, transformed with such
vector.

5 This invention also relates to a recombinant DNA
molecule comprising the Streptomyces gal operon galE, galT
or galK gene, or any functional derivative thereof. By
"functional derivative" is meant any derivative
of the Streptomyces gal operon galE, galT or galK gene
which functions in substantially the same way as the
naturally occurring gene in terms of production of an
10 active galE, galT, or galK type gene product. Such
derivatives include partial sequences of the Streptomyces
gal operon galE, galT, or galK gene, as well as
derivatives produced by modification of the gal operon
sequence. Techniques for effecting such modification are
15 known in the art, and some have been outlined above. The
naturally occurring Streptomyces gal operon galE, galT
and/or galK gene can be isolated from the naturally
occurring Streptomyces gal operon by conventional
techniques. The Streptomyces gal operon galE, galT and/or
20 galK gene can be used as a selection marker in an
appropriate host mutant. By "appropriate host mutant is
meant a host which does not utilize galactose because it
contains a defect within a homologous galE, galT and/or
galK gene. Thus, a recombinant DNA molecule (comprising
25 the Streptomyces gal operon galE, galT and/or galK gene
and a foreign functional DNA sequence, both of which are
operatively linked to appropriate regulatory region),
which can be prepared by conventional techniques, can be
transformed into an appropriate host mutant by
30 conventional techniques for incorporation into the host
genome by homologous recombination to enable detection of
transformants without the need of maintaining an expensive
antibiotic selection. Likewise, a recombinant DNA vector
comprising the Streptomyces gal operon galE, galT and/or
35 galK gene and a foreign functional DNA sequence, both of
which are operatively linked to appropriate regulatory

1 regions, as well as a replicon, can be transformed into an
appropriate host mutant by conventional techniques to
enable detection of transformants without the need of
maintaining an expensive antibiotic selection. The
5 Streptomyces gal operon galE, galK and/or galT gene is
also useful for complementation of an appropriate host
mutant.

The Streptomyces gal operon galE gene is also
useful for providing a ribosome binding site and
10 initiation codon which can be fused to a foreign
functional DNA sequence to enable the expression of such
coding sequence when incorporated into an appropriate
expression vector and transformed into an appropriate
host. If such foreign functional DNA sequence is fused to
15 the galE gene ribosome binding site and initiation codon
in a recombinant DNA expression vector comprising the
Streptomyces gal operon P2 promoter expression unit, or
the entire gal operon, such DNA sequence will be
constitutively expressed when such vector is transformed
20 into an appropriate host organism. If such DNA sequence
is fused to the galE gene ribosome binding site and
initiation codon in a recombinant DNA expression vector
comprising the Streptomyces gal operon P2 promoter
regulated region, expression of such DNA sequence can be
25 regulated when such vector is transformed into an
appropriate host organism by controlling the presence or
absence of galactose or glucose.

The Streptomyces gal operon galT gene is also
useful for providing a ribosome binding site and
30 initiation codon which can be fused to a foreign
functional DNA sequence to enable the expression of such
coding sequence when incorporated onto an appropriate
expression vector and transformed into an appropriate
host. If such DNA sequence is fused to the galT gene
35 ribosome binding site and initiation codon in a
recombinant DNA expression vector comprising the

1 Streptomyces gal operon P1 promoter r gulated region, or
the entir gal op ron, expression of such coding sequence
can be regulated in a host transformed with such vector as
outlined above.

5 This invention also relates to a recombinant DNA
vector comprising a replicon, Streptomyces gal operon, or
a functional and regulatable derivative thereof, and a
foreign functional DNA sequence operatively linked to such
operon. Such vector can be prepared by conventional
10 techniques. The replicon employed should be one known for
its ability to stably and extrachromosomally, maintain a
vector in the host organism which is to be the host
transformed with the vector.

15 This invention also relates to a transformed host
microorganism comprising a recombinant DNA vector wherein
said vector contains a replicon, the Streptomyces gal
operon, or a functional and regulatable derivative
thereof, and a foreign functional DNA sequence operatively
linked to such operon; and to the method of preparing such
20 host which comprises transforming an appropriate host
microorganism with such vector. Appropriate host
microorganisms which may be employed in the method of this
invention include viruses, and eukaryotic and prokaryotic
cells or organisms, especially actinomycetes, such as
25 those of the genus Streptomyces. The most preferred host
microorganisms belong to the genus Streptomyces.

Preferred species of Streptomyces include Streptomyces
lividans, S. coelicolor, S. azureus and S. albus.

30 Transformation of such host microorganism with such vector
can be accomplished using conventional techniques such as
the method of Chater et al., Curr. Top. Micro. Imm., 96,
69-95 (1982). This invention also related to a method of
expressing the functional DNA sequence contained by such
transformed host of this invention which comprises
35 cultivating such transformed host under suitable
conditions such that the functional DNA sequence is

1 expressed. By "suitable conditions" is meant those
conditions which will allow the host to grow and which
enable the expression of the functional DNA sequence.
Such suitable conditions can be determined by one of skill
5 in the art using conventional techniques and will depend
on various factors, such as the host organism employed and
the functional DNA sequence to be expressed. This
invention is also related to a method of regulating the
expression of the functional DNA sequence contained by
10 such transformed host which comprises cultivating a
transformed host containing such functional DNA sequence
under appropriate conditions such that its expression is
regulatable. By "appropriate conditions" is meant those
conditions which enable the Streptomyces gal operon (and
15 thus the foreign functional DNA sequence) to be
regulatable. By "regulatable" is meant responsive to the
presence of galactose or its metabolites and the presence
of glucose or its metabolites in the growth media of the
transformed host cell. Such regulation can be carried out
20 by addition or deletion of galactose or glucose to the
transformed host's culture medium. The optimal levels of
galactose and/or glucose for up or down-regulation of the
expression of the foreign functional DNA coding sequence
by the transformed host of this invention can be readily
25 determined by one of skill in the art using conventional
techniques.

This invention also relates to a recombinant DNA
vector comprising a replicon, a Streptomyces gal operon P2
30 promoter expression unit, or a functional derivative
thereof, and a foreign functional DNA sequence operatively
linked to such unit. Such a vector can be prepared by
conventional techniques. The replicon employed should be
one known for its ability to stably, and extra-
35 chromosomally, maintain a vector in the host organism
which is to be transformed with the vector.

1 This invention also relates to a transformed host
microorganism comprising a recombinant DNA vector wherein
said vector contains a replicon, the Streptomyces gal
operon P2 promoter expression unit, or a functional
5 derivative thereof, and a foreign functional DNA sequence
operatively linked to such unit; and to the method of
preparing such host which comprises transforming an
appropriate host microorganism with such vector. By the
term "operatively linked" is meant that a functional DNA
10 sequence is transcriptionally or translationally linked to
an expression control sequence (i.e., the Streptomyces gal
operon, P2 promoter expression unit, P1 promoter regulated
region, P1 promoter or P2 promoter) in such a way so that
the expression of the functional DNA sequence is under
15 control of the expression control sequence. Thus, for
example, a foreign functional DNA sequence can be
transcriptionally or translationally linked to the
Streptomyces gal operon by inserting such operon within
the Streptomyces gal operon P1 or P2 promoter transcript.
20 By the term "replicon" is meant that region of DNA on a
plasmid which functions to maintain, extrachromosomally,
such plasmid in a host microorganism or cell transformed
therewith. It has also been discovered that the
Streptomyces gal operon, and smaller portions thereof, is
25 useful as a nucleic acid probe to obtain homologous
sequences from other cells and organisms. Appropriate
host microorganisms which may be employed in the method of
this invention include any virus or eukaryotic or
prokaryotic cell or organism, especially any actinomycetes
30 such as those of the genus Streptomyces. The most
preferred host microorganisms belong to the genus
Streptomyces. Preferred species of Streptomyces include
Streptomyces lividans, S. coelicolor, S. azureus and S.
albus. Transformation of such host microorganism with
35 such vector can be accomplished using conventional

1 techniques such as the method of Chater et al., Curr. Top.
2 Micro. Imm., 96, 69-95 (1982). This invention also
3 related to a method of expressing the functional DNA
4 sequence contained by such transformed host of this
5 invention which comprises cultivating such transformed
6 host under suitable conditions such that the functional
7 DNA sequence is expressed. By "suitable conditions" is
8 meant those conditions which will allow the host to grow
9 and which enable the expression of the functional DNA
10 sequence. Such suitable conditions can be determined by
11 one of skill in the art using conventional techniques and
12 will depend on various factors, such as the host organism
13 employed and the functional DNA sequence to be expressed.

14 This invention also relates to a recombinant DNA
15 vector comprising a replicon, a Streptomyces gal operon P1
16 promoter regulated region, or a functional and regulatable
17 derivative thereof, and a foreign functional DNA sequence
18 operatively linked to such region. Such a vector can be
19 prepared by conventional techniques. The replicon
20 employed should be one known for its ability to stably and
21 extrachromosomally maintain a vector in the host organism
22 which is to be the host transformed with the vector.

23 This invention also relates to a transformed host
24 microorganism comprising a recombinant DNA vector wherein
25 said vector contains a replicon, a Streptomyces gal operon
26 P1 promoter regulated region, or a functional and
27 regulatable derivative thereof, and a foreign functional
28 DNA sequence operatively linked to such region; and to the
29 method of preparing such host which comprises transforming
30 an appropriate host microorganism with such vector.
31 Appropriate host microorganisms which may be employed
32 include any virus or eukaryotic or prokaryotic cell or
33 organism especially actinomycetes such as those of the
34 genus Streptomyces. The most preferred host
35 microorganisms belong to the genus Streptomyces.

1 Preferred species of Streptomyces include Streptomyces
2 lividans, S. coelicolor, S. azureus and S. albus.

3 Transformation of such host microorganism with such vector
4 can be accomplished using conventional techniques such as
5 the method of Chater et al., Curr. Top. Micro. Imm., 96,
6 69-95 (1982). This invention also related to a method of
7 expressing the foreign functional DNA sequence contained
8 by such transformed host of this invention which comprises
9 cultivating such transformed host under suitable
10 conditions such that the functional DNA sequence is
11 expressed. By "suitable conditions" is meant those
12 conditions which will allow the host to grow and which
13 enable the expression of the functional DNA sequence.

14 Such suitable conditions can be determined by one of skill
15 in the art using conventional techniques and will depend
16 on various factors, such as the host organism employed and
17 the functional DNA sequence to be expressed. This
18 invention also related to a method of regulating the
19 expression of the functional DNA sequence contained by
20 such transformed host which comprises cultivating a
21 transformed host containing such functional DNA sequence
22 under appropriate conditions such that its expression is
23 regulatable. By "appropriate conditions" is meant those
24 conditions which enable the Streptomyces gal operon P1
25 promoter regulated region (and thus the foreign functional
26 DNA sequence) to be regulatable. By "regulatable" is
27 meant responsive to the presence or absence of galactose
28 or its metabolites and the presence or absence of glucose
29 or its metabolites in the growth media of the transformed
30 host cell. Such regulation can be carried out by addition
31 or deletion of galactose or glucose to the transformed
32 host's culture medium.

33 This invention also relates to a recombinant DNA
34 vector comprising a replicon, a Streptomyces gal operon P2
35 promoter, or a functional derivative thereof, and a
36 foreign functional DNA sequence operatively linked to such

1 promoter. Such a vector can be prepared by conventional
techniques. The replicon employed should be one known for
its ability to stably and extrachromosomally maintain a
vector in the host organism which is to be the host
5 transformed with the vector.

This invention also relates to a transformed host
microorganism comprising a recombinant DNA vector wherein
said vector contains a replicon, a Streptomyces gal operon
P2 promoter, or a functional derivative thereof, and a
10 foreign functional DNA sequence operatively linked to such
region; and to the method of preparing such host which
comprises transforming an appropriate host microorganism
with such vector. Appropriate host microorganisms which
may be employed include actinomycetes such as those of the
15 genus Streptomyces. The most preferred host
microorganisms belong to the genus Streptomyces.
Preferred species of Streptomyces include Streptomyces
lividans, S. coelicolor, S. azureus and S. albus.
Transformation of such host microorganism with such vector
20 can be accomplished using conventional techniques such as
the method of Chater et al., Curr. Top. Micro. Imm., 96,
69-95 (1982). This invention also related to a method of
expressing the foreign functional DNA sequence contained
by such transformed host of this invention which comprises
25 cultivating such transformed host under suitable
conditions such that the functional DNA sequence is
expressed. By "suitable conditions" is meant those
conditions which will allow the host to grow and which
enable the expression of the functional DNA sequence.
30 Such suitable conditions can be determined by one of skill
in the art using conventional techniques and will depend
on various factors, such as the host organism employed and
the functional DNA sequence to be expressed.

This invention also relates to a recombinant DNA
35 vector comprising a replicon, Streptomyces gal operon P1
promoter, or any regulatable and functional derivative

1 thereof, and a foreign functional DNA sequence operatively
link d to such region. Such a vector can be prepared by
conventional techniques. The replicon employed should be
one known for its ability to stably and extrachromosomally
5 maintain a vector in the host organism which is to be the
host transformed with the vector.

This invention also relates to a transformed host
microorganism comprising a recombinant DNA vector wherein
said vector contains a replicon, the Streptomyces gal
10 operon P1 promoter, or any regulatable and functional
derivative thereof, and a foreign functional DNA sequence
operatively linked to such region; and to the method of
preparing such host which comprises transforming an
appropriate host microorganism with such vector.

15 Appropriate host microorganisms which may be employed
include viruses or prokaryotic or eukaryotic cells or
organisms, especially actinomycetes such as those of the
genus Streptomyces. The most preferred host
microorganisms belong to the genus Streptomyces.

20 Preferred species of Streptomyces include Streptomyces
lividans, S. coelicolor, S. azuraeus and S. albus.
Transformation of such host microorganism with such vector
can be accomplished using conventional techniques such as
the method of Chater et al., Curr. Top. Micro. Imm., 96,
25 69-95 (1982). This invention also relates to a method of
expressing the foreign functional DNA sequence contained
by such transformed host of this invention which comprises
cultivating such transformed host under suitable
conditions such that the functional DNA sequence is
30 expressed. By "suitable conditions" is meant those
conditions which will allow the host to grow and which
enable the expression of the functional DNA sequence.
Such suitable conditions can be determined by one of skill
in the art using conventional techniques and will depend
35 on various factors, such as the host organism employed and

1 the foreign functional DNA sequence to be expressed. This
invention also relates to a method of regulating the
expression of the functional DNA sequence contained by
such transformed host which comprises cultivating a
5 transformed host containing such foreign functional DNA
sequence under appropriate conditions such that its
expression is regulatable. By "appropriate conditions" is
meant those conditions which enable the gal operon P1
promoter (and thus the functional DNA sequence) to be
10 regulatable. By "regulatable" is meant responsive to the
presence or absence of galactose or its metabolites and
the presence of glucose or its metabolites in the growth
media of the transformed host cell. Such regulation can
be carried out by addition or deletion of galactose or
15 glucose to the transformed host's culture medium.

EXAMPLES

In the following Examples, specific embodiments
of the invention are more fully disclosed. These Examples
are intended to be illustrative of the subject invention
20 and should not be construed as limiting its scope. In all
Examples, temperature is in degrees Centigrade (°C).

By utilizing conventional methods, such as those
outlined in the following Examples, one of skill in the
art can isolate the gal operon from any galactose
25 utilizing strain of Streptomyces. Furthermore, by
utilizing techniques similar to those employed herein to
isolate the Streptomyces gal operon, one of skill in the
art can attempt to use the Streptomyces gal operon to
isolate a gal operon from other galactose utilizing other
30 strains of Streptomyces, especially S. coelicolor, S.
azuraeus, S. albus and other S. lividans strains.

Molecular genetic manipulations and other
techniques employed in the following Examples are
described in Hopwood et al., Genetic Manipulation of
35 Streptomyces: A Laboratory Manual, John Innes Foundation,
Norwich, England (1985).

1

ABBREVIATIONS

In the following Examples, the following abbreviations may be employed:

5

LB: 10 grams (g) tryptone, 5 g yeast extract, 5g

NaCl

MBSM (modified MBSM): See, Brawner et al., Gene, 40, 191 (1985) (in press)

10

MOPS: (3)-N-morpholino-(propane-sulfonic acid)

YEME + $MgCl_2$ + Glycine: [per liter(l)] 3 g yeast extract, 5 g peptone, 3 g malt extract, 10 g glucose, 10 g $MgCl_2 \cdot 6H_2O$, 340 g sucrose.

15

SL: Mix together $(NH_4)_2SO_4$ (1g/l); L-asparagine (2 g/l); K_2HPO_4 (9 g/l); NaH_2PO_4 (1 g/l) for 0.2% agar and autoclave. Then mix with yeast extract (20 g/l), $MgCl_2$ (5 g/l); $CuCl_2$ (0.1 g/l); Trace elements [20 ml/l - include $ZnCl_2$ -40 mg/l; $FeCl_3 \cdot 6H_2O$ (200 mg/l); $CuCl_2 \cdot 2H_2O$ (10 mg/l); $NaB_4O_7 \cdot 10H_2O$ (10 mg/l); $(NH_4)_6MO_7O_{24} \cdot 4H_2O$ (10 mg/l)] filter and sterilize.

20

YEME (Ym base): (per liter) yeast extract (3g); peptone (5g); malt extract (3g); $MgCl_2 \cdot 6H_2O$ (2g)

25

Ymglu: YEME + glucose (10g)

Ymgal: YEME + galactose (10g)

30

35

1

BACTERIAL STRAINS

In the following Examples, the following strains of E. coli are employed:

5

CGSC Strain #(a)	Strain Designation	Sex	Chromosomal Markers
4473 (<u>galE</u> ⁻)	W3109	F ⁻	<u>galE9</u> , ^(b) g ⁻ ;IN(rrnD-rrnE)1
4467 (<u>galT</u> ⁻)	W3101	F ⁻	<u>galT22</u> ^(b) g ⁻ ;IN(rrnD-rrnE)1
10 4498 (<u>galE</u> ⁻)	PL-2	Hfr	<u>thi-1</u> , <u>relA1</u> , <u>921E28</u> ,g ⁻ , <u>spoT1</u>

(a) CGSC Strain # is the stock number designated for such strain by the E. coli Genetic Stock Center of the Department of Human Genetics, Yale University School of Medicine, 333 Cedar Street, P.O. Box 3333, New Haven, Connecticut, 06510, U.S.A.

15

(b) galE9 is the old Lederberg gal9; galT22 is the old Lederberg gal₁.

20

S1 ANALYSIS

S1 analysis is used to identify the 5' end of RNAs and the length of a RNA of interest. In the following Examples, S1 analysis refers to S1 experiments carried out according to the method of Weaver et al., Nucl. Acids Res., 7, 1175 (1979) and Berk et al., Proc. Natl. Acad. Sci. USA, 75, 1214 (1978).

25

EXAMPLE I

A. CLONING OF A STREPTOMYCES LIVIDANS GALACTOKINASE GENE.

30

Streptomyces lividans strain 1326 is described by Bibb et al., Mol. Gen. Genetics, 184, 230-240 (1981) and was obtained from D. A. Hopwood, John Innes Foundation, Norwich, England. Streptomyces lividans strain 1326 and S. lividans strain 1326 containing the pIJ6 plasmid were deposited in the Agricultural Research Culture Collection,

35

1 Peoria, Illinois, U.S.A., on June 1, 1982, under accession numbers NRRL 15091 and 15092, respectively.

5 High molecular weight chromosomal DNA was isolated from Streptomyces lividans strain 1326 according to the method of Maniatis et al., "Molecular Cloning. A Laboratory Manual", Cold Spring Harbor Laboratory (1982) and was size fractionated on a 10-40% sucrose gradient (See, Maniatis et al., cited above, p. 284-285).

10 Fractions of 18-24 kilobase (Kb) pairs were combined and dialyzed exhaustively against 10 mM Tris-HCl/1 mM EDTA (pH 8). Cosmid shuttle vector pJW357 was employed to clone such fractionated chromosomal DNA in its entirety. pJW357 was constructed by fusing pDPT6 cut with PstI to pIJ350 cut with PstI. pIJ350 is described in Kieser et al., Mol. Gen. Genet., 185, 223-238 (1982). pDPT6 is a tetracycline and chloramphenicol resistant, pBR322-based E. coli cosmid cloning vector described in Taylor et al., U.S. Patent No. 4,476,227. pJW357 has a unique EcoRI site in the
20 chloramphenicol resistance gene and a unique BamHI site in the Tc^R (tetracycline) resistance gene. pJW357 was digested with BamHI, dephosphorylated with alkaline phosphatase, and ligated to the fractionated chromosomal DNA described above.

25 The ligation product was packaged into bacteriophage heads (using the in vitro packaging system described by Maniatis et al., cited above, p. 264-265) and transfected into E. coli strain K21 which is a galK⁻ derivative of E. coli MM294. The transformation culture
30 was grown for two hours in LB and for an additional two hours in LB with 25 ug/ml chloramphenicol, washed three times with equal volumes of M9 media [see, Miller, "Experiments in Molecular Genetics", Cold Spring Harbor Laboratory (1972)] without a carbon source, and plated
35 onto M9 agar [supplemented with proline, histidine, arginine, isoleucine, leucine, saline and .5% galactose;

1 See, Adams et al., Biochem. Biophys. Res. Comm., 89(2),
650-58 (1979)] with 30 mg/ml chloramphenicol. Twenty
plates were spread with approximately 200 transformants
per plate. After three days incubation at 37°C, no
5 transformants were detected. The minimal plates were then
sprayed with nicotinic acid to 5 ug/ml to supplement the
nicotinic acid requirement of E. coli strain K21, and the
incubation was continued for 3 more days at 37°C and for 2
additional days at room temperature. After such
10 incubation, the surviving colonies were patched to both
MacConkey galactose agar (MAC-GAL) [See, Miller et al.,
cited above] with 30 ug/ml chloramphenicol and to M63
minimal agar [See, Miller et al., cited above]
supplemented with .5% galactose, 5 ug/ml nicotinic acid, 5
15 ug/ml thiamine and 30 ug/ml chloramphenicol. Only two
colonies contained cosmid DNA that transformed E. coli K21
to a galK⁺ phenotype. Such cosmids were designated as
pSLIVGAL-1 and pSLIVGAL-2. Both colonies were light red
on MAC-GAL (i.e., they were galK⁺) and also grew on the
20 M63 medium.

Plasmids pSLIVGAL-1 and pSLIVGAL-2 were isolated
from the two galK⁺ colonies described above and were
transformed, according to the method of Chater et al.,
Curr. Top. Micro. Imm., 96, 69-95 (1982), into Streptomyces
25 lividans strain 1326-12K (a galK deficient strain isolated
after UV mutagenesis of S. lividans strain 1326, See,
Brawner et al., Gene, 40, 191 (1985), (in press). Plasmid
encoded complementation of the S. lividans 1326-12K
(galK⁻) host was tested by observing growth of spores
30 plated on MBSM-gal-thiostrepton according to the method of
Brawner et al., Gene, 40, 191 (1985) (in press).
pSLIVGAL-2 showed no detectable complementation of the
Streptomyces 1326-12K host.

Cell extracts were prepared from cultures grown
35 in SL medium supplemented with 1% glucose or galactose and
10 %g/ml thiostrepton. The extracts were analyzed for

1 galactokinase production by immunoblot analysis (see,
Brawner et al., Gene, 40, 191 (1985), in press) using
rabbit antisera prepared against E. coli galactokinase.
5 The protein detected by immunoblot analysis was the
approximate size of E. coli galK. Such protein appeared
in galactose supplemented cultures of Streptomyces at
levels several fold higher than in glucose cultures.

10 B. MAPPING OF THE S. LIVIDANS GALK REGION WITHIN A COSMID.

The galK region of the pSLIVGAL1 and pSLIVGAL2
cosmids, prepared as described above, was identified by
cloning random fragments from the cosmids into a pUC18
derivative [See, Norrander et al., Gene, 26, 101-106
15 (1983)] and scoring complementation of E. coli strain
MM294 (galK⁻) on MAC-GAL medium. The cosmid clone was
partially digested with Sau3AI (using conditions which
maximized the yield of 2 to 4 kilobase fragments), and the
products of this reaction were ligated into the BglII site
20 of pUC18-TT6, a derivative of pUC18 constructed by
insertion of the following synthetic DNA sequence into the
BamHI site of pUC18:

5'GATCAGATCTTGATCACTAGCTAGCTAG 3'

3' TCTAGAACTAGTGATCGATCGATCCTAG 5'

25

Twelve galK⁺ clones (red on MAC-GAL) were screened for
size. One clone, designated as plasmid pSAU10, was the
smallest and had an insert size of approximately 1.4 Kb.

30 In contrast to colonies containing pSLIVGAL1, the
pUC clones were very red on MAC-GAL medium, indicating an
increased production of galactokinase. The most likely
explanation for the increased enzyme level was that the S.
lividans galK gene was now being transcribed by an E. coli
35 promoter which was stronger than the upstream promoter on
the cosmid.

1

The insert of pSAU10 was isolated as an EcoRI to HindIII fragment (these sites flank the insert region of pUC18-TT6) for use as a probe for the S. lividans galK gene. The chromosomal DNA used in the cloning was restricted with EcoRI plus MluI and BamHI plus BglII, and then blotted according to the method of Southern, J. Mol. Biol., 98, 503 (1975). The pSAU10 fragment was nick translated and hybridized to the blot. The probe identified a 1.3 kb EcoRI-MluI fragment and a 5 kb BamHI-BglII fragment in the chromosomal digests. When this data was compared to the map of the cosmid insert, the location of the galK gene (between map positions 5 and 7, See Table A) was confirmed.

15

C. DNA SEQUENCING OF THE S. LIVIDANS GAL OPERON.

The Streptomyces lividans gal operon was sequenced by chain termination [(See, Sanger et al., Proc. Nat'l Acad. Sci., U.S.A., 74, 5463 (1977))] and chemical cleavage [See, Maxam and Gilbert, Methods in Enzymology, 65, 499 (1980)]. The initial sequences of galK were derived from Sau3AI and SalI fragments of the insert of pSAU6 (a 2.3 Kb sibling of pSAU10) shotgun cloned into the BamHI and SalI sites (respectively) of M13 mp 10 [See, Messing, Methods in Enzymology, 101, 20 (1983)]. Amino acid sequences of the S. lividans galT, galE and galK genes were predicted by computer, and further analyzed by comparison with amino acid sequences of the E. coli and or S. cerevisiae galactokinase, gal-1-phosphate uridylyltransferase and UDP-4-epimerase enzymes. The sequences of these proteins were predicted by computer analysis using the total or partial DNA sequence of the genes which encode the gal enzymes [see, Debouck et al., Nuc Acids. Res., 13(6), 1841-1853 (1985), and Citron and Donelson, J. Bacteriology, 158, 269 (1984)]. Some homology was found between the inferred protein sequence

35

1

for the S. lividans galK, galT, galE gene products and their respective E. coli and/or S. cerevisia gene products.

5

The complete DNA sequence of the S. lividans gal operon is shown in Table 1. Included in Table 1 are the transcription start sites for the operon's promoters and the predicted amino acid sequences of the galT, galE and galK gene products.

10

15

20

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-35-

TABLE 1
TRANSLATED SEQUENCE OF STREPTOMYCES LIVIDANS
GALACTOSE OPERON

	-120	-110	-100	-90	-80	-70													
	CTA	CGC	CTC	CGC	GTT	CAG	TAA	TTC	AAC	ACT	TTT	GGT	GAT	GAA	CTT	TGT	TTG	ATT	GTG
10		-60		-50		-40		-30		-20									
	ATG	TGA	CAG	GGG	GGT	GGT	GGG	TTG	TGA	TGT	GTT	ATG	TTT	GAT	TGT	GTT	GGA	TGA	TTG
																galP1			
15	-10		1		10		20		30		40								
	ACG	GGC	GTG	CTG	GTG	ACT	CAT	GGG	TGG	GTG	CAG	AGG	AGT	GGG	GCA	GTG	AAG	AAG	ACC
					Met	Thr	His	Gly	Trp	Val	Gln	Arg	Ser	Ala	Ala	Val	Lys	Lys	Thr
					galT														
	50		60		70		80		90		100								
	TCG	ACC	CGG	CTG	GCC	GAC	GGC	CGT	GAG	CTG	GTC	TAC	TAC	GAC	CTG	CGC	GAC	GAC	ACC
	Ser	Thr	Arg	Leu	Ala	Asp	Gly	Arg	Glu	Leu	Val	Tyr	Tyr	Asp	Leu	Arg	Asp	Asp	Thr
20		110		120		130		140		150									
	GTG	CGC	GAC	GCC	GTG	GAC	CGC	CGT	CCG	CTG	GAG	CGG	ACC	GTG	ACC	ACG	TCC	GAG	GTG
	Val	Arg	Asp	Ala	Val	Asp	Arg	Arg	Pro	Leu	Glu	Arg	Thr	Val	Thr	Thr	Ser	Glu	Val
25	160		170		180		190		200		210								
	CGA	CGC	GAC	CCG	CTG	CTC	GGC	GAC	TCC	GCG	CCG	TCC	CGC	CTC	GCA	CCG	GCA	GGG	GGC
	Arg	Arg	Asp	Pro	Leu	Leu	Gly	Asp	Ser	Ala	Pro	Ser	Arg	Leu	Ala	Pro	Ala	Gly	Ala
	220		230		240		250		260		270								
	CAC	CTA	CCA	TCC	GCC	GGC	CGA	CCA	GTG	CCC	GCT	GTG	CCG	GTG	GGA	CGG	GGA	ACG	GCT
	His	Leu	Pro	Ser	Ala	Gly	Arg	Pro	Val	Pro	Ala	Val	Pro	Val	Gly	Arg	Gly	Thr	Ala
30		280		290		300		310		320		330							
	GAG	CGA	GAT	CCG	GCC	TAT	GAC	GTG	GTG	GTC	TTC	GAG	AAT	CGC	TTT	CCC	TCC	CTG	GCC
	Glu	Arg	Asp	Pro	Ala	Tyr	Asp	Val	Val	Val	Phe	Glu	Asn	Arg	Phe	Pro	Ser	Leu	Ala

Table 1 - (cont'd)

-36-

	340	350	360	370	380
5	GGT GAC TCC GGG CGC TGC GAG GTC GTC TGC TTC ACC TCC GAC CAC GAC GCC TCC TTC				
	Gly Asp Ser Gly Arg Cys Glu Val Val Cys Phe Thr Ser Asp His Asp Ala Ser Phe				
	390	400	410	420	430
	GCC GAC CTG ACC GAG GAG CAG GCC CGG CTG GTC GTC GAC GCC TGG ACC GAC CGC ACC				
	Ala Asp Leu Ser Glu Glu Gln Ala Arg Leu Val Val Asp Ala Trp Thr Asp Arg Thr				
10	450	460	470	480	490
	TCC GAG CTG TCC CAT CTG CCC TCC GTT GAA CAG GTG TTC TGC TTC GAG AAC CGG GGC				
	Ser Glu Leu Ser His Leu Pro Ser Val Glu Gln Val Phe Cys <u>Phe</u> <u>Glu</u> <u>Asn</u> <u>Arg</u> <u>Gly</u>				
	510	520	530	540	550
15	GCC GAG ATC GGG GTG ACG CTG GGT CAC CCG CAC GGG CAG ATC TAC GCC TAC CCG TTC				
	<u>Ala</u> Glu <u>Ile</u> <u>Gly</u> Val Thr Leu Gly <u>His</u> <u>Pro</u> <u>His</u> <u>Gly</u> <u>Gln</u> Ile Tyr Ala Tyr Pro Phe				
	560	570	580	590	600
	ACC ACC CCC CGC ACC GCC CTG ATG CTC CGT TCA CTC GCC GCC CAC AAG GAC GCG ACG				
	Thr Thr Pro Arg Thr Ala Leu Met Leu Arg Ser Leu Ala Ala His Lys Asp Ala Thr				
20	620	630	640	650	660
	GCC GGG GGG AAC CTG TTC GAC TCC GTG CTG GAG GAG GAG CTG GCC GGT GAG CGG GTC				
	Gly Gly Gly Asn Leu Phe Asp Ser Val Leu Glu Glu Glu Leu Ala Gly Glu Arg Val				
	680	690	700	710	720
25	GTC CTG GAG GGT GAG CAC TGG GCC GCC TTC GTC GCG TAC GGC GCG CAC TGG CCG TAC				
	Val Leu Glu Gly Glu His Trp Ala Ala Phe Val Ala Tyr Gly Ala His Trp Pro Tyr				
	730	740	750	760	770
	GAG GTG CAC CTC TAC CCG AAG CGG CGG GTG CCC GAT CTG CTC GGG CTC GAC GAG GCG				
	Glu Val His Leu Tyr Pro Lys Arg Arg Val Pro Asp Leu Leu Gly Leu Asp Glu Ala				
30	790	800	810	820	830
	GCT CGC ACA GAA TTC CCC AAG GTC TAC CTG GAG CTG CTG AGG CGT TTC GAC CGG ATC				
	Ala Arg Thr Glu Phe Pro Lys Val Tyr Leu Glu Leu Leu Arg Arg Phe Asp Arg Ile				

35

1 table 1 - (cont'd)

-37-

		850		860		870		880		890		900									
5		TTC	GGC	GAG	GGC	GAG	CCC	CCG	ACC	CCC	TAC	ATC	GGC	GCC	TGG	CAC	CAG	GGC	CCG	TTC	
		Phe	Gly	Glu	Gly	Glu	Pro	Pro	Thr	Pro	Tyr	Ile	Ala	Ala	Trp	His	Gln	Ala	Pro	Phe	
		910		920		930		940		950											
		GGG	CAG	CTG	GAG	TTC	GAG	GGT	GTG	ACG	CGC	GAC	GAC	TTC	GGC	CTC	CAC	CTG	GAA	CTT	
		Gly	Gln	Leu	Glu	Phe	Glu	Gly	Val	Thr	Arg	Asp	Asp	Phe	Ala	Leu	His	Leu	Glu	Leu	
10		960		970		980		990		1000		1010									
		TTC	ACT	TCC	CCC	GTA	CGT	CCG	GCA	AGC	TGA	AGT	TCC	TCG	CCG	GCT	CCG	AAT	CCG	GCA	
		Phe	Thr	Ser	Ala	Val	Arg	Pro	Ala	Ser	---				galP2						
		1020		1030		1040		1050		1060		1070									
15		TGA	ACG	TGTT	CAT	CAA	CGAC	GT	ACCC	CCG	GAG	CGCG	CGG	CCG	GAG	CGC	ACT	GCG	GAG	GTAG	CGAG
		1080		1090		1100		1110		1120		1130									
		TTC	ATG	AGC	GGG	AAG	TAC	CTG	GTG	ACA	GGT	GGT	GCC	GGA	TAC	GTC	GGC	AGC	GTC	GTC	
		Met	Ser	Gly	Lys	Tyr	Leu	Val	Thr	Gly	Gly	Ala	Gly	Tyr	Val	Gly	Ser	Val	Val		
		galE																			
20		1140		1150		1160		1170		1180		1190									
		GGC	CAG	CAC	TTG	GTG	GAG	GGC	GGG	AAC	GAG	GTC	GTG	GTG	CTG	CAC	AAT	CTC	TCG	ACC	
		Ala	Gln	His	Leu	Val	Glu	Ala	Gly	Asn	Glu	Val	Val	Val	Leu	His	Asn	Leu	Ser	Thr	
		1200		1210		1220		1230		1240											
25		GGC	TTC	CGT	GAG	GTG	TGC	CGG	CGG	GTG	CCT	CGT	TCG	TCG	AGC	CGA	CAT	CCG	GGA	CGC	
		Gly	Phe	Arg	Glu	Val	Cys	Arg	Arg	Val	Pro	Arg	Ser	Ser	Arg	Arg	His	Pro	Gly	Arg	
		1250		1260		1270		1280		1290		1300									
		CGC	CAA	GTG	CGT	GGA	CGG	CTC	TCG	TTC	GAC	GGC	GTG	CTG	CAC	TTC	CCC	CCC	TTC	TCC	
		Arg	Gln	Val	Arg	Gly	Arg	Leu	Ser	Phe	Asp	Gly	Val	Leu	His	Phe	Ala	Ala	Phe	Ser	
30		1310		1320		1330		1340		1350		1360									
		CAG	GTC	GGC	GAG	TCG	GTC	GTG	AAG	CCC	GAG	AAG	TAC	TGG	GAC	AAC	AAC	GTC	GGT	GGC	
		Gln	Val	Gly	Glu	Ser	Val	Val	Lys	Pro	Glu	Lys	Tyr	Trp	Asp	Asn	Asn	Val	Gly	Gly	

35

1 Label 1 - (cont'd)

-38-

1370 1380 1390 1400 1410 1420
 ACC ATG GCG CTG CTG GAG GCC ATG CCG GCG GGT GTG CCG CCG CTC GTC TTC TCC
 Thr Met Ala Leu Leu Glu Ala Met Arg Gly Ala Gly Val Arg Arg Leu Val Phe Ser
 5
 1430 1440 1450 1460 1470
 TCC ACG GCC GCC ACC TAC GGC GAG CCC GAG CAG GTT CCC ATC GTC GAG TCC GCG CCG
 Ser Thr Ala Ala Thr Tyr Gly Glu Pro Glu Gln Val Pro Ile Val Glu Ser Ala Pro
 10 1480 1490 1500 1510 1520 1530
 ACG AGG CCC ACC AAT CCG TAC GGC GCC TCG AAG CTC GCC GTC GAC CAC ATG ATC ACC
 Thr Arg Pro Thr Asn Pro Tyr Gly Ala Ser Lys Leu Ala Val Asp His Met Ile Thr
 1540 1550 1560 1570 1580 1590
 GGC GAG GCG GCG GCC CAC GGG CTG GGC GCG GTC TCC GTG CCG TAC TTC AAC GTC GCG
 Gly Glu Ala Ala Ala His Gly Leu Gly Ala Val Ser Val Pro Tyr Phe Asn Val Ala
 15
 1600 1610 1620 1630 1640
 GGC GCG TAC GGC GAG TAC GGC GAG CCG CAC GAC CCC GAG TCG CAT CTG ATT CCG CTG
 Gly Ala Tyr Gly Glu Tyr Gly Glu Arg His Asp Pro Glu Ser His Leu Ile Pro Leu
 20 1650 1660 1670 1680 1690 1700
 GTC CTT CAA GTG GCG CAG GGC AGG CCG GAG GCC ATC TCC GTC TAC GGC GAC GAC TAC
 Val Leu Gln Val Ala Gln Gly Arg Arg Glu Ala Ile Ser Val Tyr Gly Asp Asp Tyr
 1710 1720 1730 1740 1750 1760
 CCG ACG CCG GAC CGA CCT GTG TGC GCG ACT ACA TCC ACG TCG CCG ACC TGG CCG AGG
 Pro Thr Pro Asp Arg Pro Val Cys Ala Thr Thr Ser Thr Ser Pro Thr Trp Pro Arg
 25 1770 1780 1790 1800 1810
 CCC ACC TGC TGG CCG TGC GCC GCC GCC CCG GCG GAG CAC CTC ATC TGC AAC CTG GGC
 Pro Thr Cys Trp Pro Cys Ala Ala Ala Pro Gly Glu His Leu Ile Cys Asn Leu Gly
 30 1820 1830 1840 1850 1860 1870
 AAC GGC AAC GGC TTC TCC GTC CCG GAG GTC GTC GAG ACC GTG CCG CCG GTG ACC GGC
 Asn Gly Asn Gly Phe Ser Val Arg Glu Val Val Glu Thr Val Arg Arg Val Thr Gly

35

1 Table 1 - (cont'd)

-39-

	1880	1890	1900	1910	1920	1930	
5	CAT CCG ATC CCC GAG ATC ATG GCC CCG CGC CGC GGG CGC GAC CCC GCG GTC CTG GTC						
	His Pro Ile Pro Glu Ile Met Ala Pro Arg Arg Gly Arg Asp Pro Ala Val Leu Val						
	1940	1950	1960	1970	1980	1990	
	GCG TCG GCC GCG ACC GCC CGC GAG AAG CTG GCG TGG AAC CCG TCC CCG GCG GAC CTC						
	Ala Ser Ala Gly Thr Ala Arg Glu Lys Leu Gly Trp Asn Pro Ser Arg Ala Asp Leu						
10	2000	2010	2020	2030	2040		
	GCC ATC GTG TCG GAC GCG TGG GAG TTG CCG CAG CGG CGC GCC GGC CAG TAG TA						
	Ala Ile Val Ser Asp Ala Trp Glu Leu Pro Gln Arg Arg Ala Gly Gln ---						
	2050	2060	2070	2080	2090	2100	
15	ACC GCA GTT ACC GGA AAG GCG AGC GGT CAG GGC ATG GGC GAG GCT GTC GGG GAA CCG						
				Met Gly Glu Ala Val Gly Glu Pro			
	2110	2120	2130	2140	2150		
	TCG GCG AGC GGT TCC GGG AGC TGT ACG GGG CGG ACC CGG AGC GGC TGT GGG CGC CGA						
20	Ser Ala Ser Gly Ser Gly Ser Cys Thr Gly Arg Ser Arg Arg Gly Cys Gly Arg Arg						
	2160	2170	2180	2190	2200	2210	
	GCG GCG CGG GAG AAC CTC ATC GGG GAG CAC ACC GAC TAC AAC GAC GGC TTC GTC ATG						
	Ala Gly Arg Glu Asn Leu Ile Gly Glu His Thr Asp Tyr Asn Asp Gly Phe Val Met						
	2220	2230	2240	2250	2260	2270	
25	CCT TCG CCC TGC CGC ACC AGG TCG CCG CCG TCT CCC GGC GCG AAC GAC GGC ATC CTG						
	Pro Ser Pro Cys Arg Thr Arg Ser Arg Pro Ser Pro Gly Ala Asn Asp Gly Ile Leu						
	2280	2290	2300	2310	2320		
	GCG CTG CAC TCG GCC GAC GTC GAC GCC GAC CCG GTC GAG CTG CCG GTC GCC GAC CTG						
30	Arg Leu His Ser Ala Asp Val Asp Ala Asp Pro Val Glu Leu Arg Val Ala Asp Leu						
	2330	2340	2350	2360	2370	2380	
	GCC CCC GCG TCG GAC AAG TCC TGG ACG GCG TAC CCC TCG GCG GTC CTG TGG GCG CTG						
	Ala Pro Ala Ser Asp Lys Ser Trp Thr Ala Tyr Pro Ser Gly Val Leu Trp Ala Leu						

35

Table 1 - (cont'd)

-40-

	2390	2400	2410	2420	2430	2440
5	CGC GAG GCC GGA CAC GAG CTC ACC GGC GCC GAC CTC CAC CTG GCC TCG ACC GTC CCG Arg Glu Ala Gly His Glu Leu Thr Gly Ala Asp Val His Leu Ala Ser Thr <u>Val</u> <u>Pro</u>					
	2450	2460	2470	2480	2490	
	TCC GGG GCG GGG CTC TCC TCC TCC GCG GCC CTG GAG GTC CGT CCC CTG GCG ATG AAC Ser <u>Gly</u> <u>Ala</u> <u>Gly</u> <u>Leu</u> <u>Ser</u> <u>Ser</u> <u>Ser</u> <u>Ala</u> Ala Leu Glu Val Arg Pro Leu Ala Met Asn					
10	2500	2510	2520	2530	2540	2550
	GAC CTG TAC GCC CTC GCG CTC CGC GGC TGG CAG CTG GCC GCG CTG TGC CAG CCG CCG Asp Leu Tyr Ala Leu Ala Leu Arg Gly Trp Gln Leu Ala Arg Leu Cys Gln Arg Ala					
	2560	2570	2580	2590	2600	2610
15	GAG AAC GTC TAC GTC GGC GCC CCC GTC GGC ATC ATG GAC CAG ACG GCG TCC GCC TGC Glu Asn Val Tyr Val Gly Ala Pro Val Gly Ile Met Asp Gln Thr Ala Ser Ala Cys					
	2620	2630	2640	2650	2660	2670
	TGC GAG GCG GGC ACG CCC TCT TCC TCG ACA CCC GCG ACC TCT CCC ACG GGC AGA TCC Cys Glu Ala Gly Thr Pro Ser Ser Ser Thr Pro Ala Thr Ser Pro Ser Gly Arg Ser					
20	2680	2690	2700	2710	2720	
	CCT TCG ACC TCG CCG CCG AGG GCA TGC GCC TGC TGG TCG TCG ACA CCC GGG TCA AGC Pro Ser Thr Ser Pro Pro Arg Gly Cys Ala Cys Trp Ser Ser Thr Pro Gly Ser Ser					
	2730	2740	2750	2760	2770	2780
25	ACT CCC ACA GCG AGG GCG AGT ACG GCA AGC GCC GCG CCG GCT GCG AGA AGG GCG CCG Thr Pro Thr Ala Arg Ala Ser Thr Ala Ser Ala Ala Arg Ala Ala Arg Arg Ala Pro					
	2790	2800	2810	2820	2830	2840
	CGC TGC TGG GCG TCG ACG CCG TGC GAC GTG CCG TAC GCC GAC CTG GAC GCG GCG CTG Arg Cys Trp Ala Ser Thr Arg Cys Asp Val Pro Tyr Ala Asp Leu Asp Ala Ala Leu					
30	2850	2860	2870	2880	2890	
	GAG CCG CTC GGC GAC GAG GAG GAG GTG CCG CCG CTG GTC CCG CAC GTG GTG ACC GAG Glu Arg Leu Gly Asp Glu Glu Glu Val Arg Arg Leu Val Arg His Val Val Thr Glu					

35

1 Table 1 - (cont'd)

-41-

	2900	2910	2920	2930	2940	2950
5	GAC GAC CGC GTC GAA CGG GTG GTC GCG CTG CTG GAG TCG GCG ACA CCC GGC GCA TCG Asp Glu Arg Val Glu Arg Val Val Ala Leu Leu Glu Ser Ala Thr Pro Gly Ala Ser					
	2960	2970	2980	2990	3000	3010
	GCG CCG TCC TGG TCG AGG GCC ACG CCT GCT GCG CGA CGA CTT CCG CAT CTC CTC CCG Ala Pro Ser Trp Ser Arg Ala Thr Pro Ala Ala Arg Arg Leu Pro His Leu Leu Pro					
10	3020	3030	3040	3050	3060	
	CGA GCT GGA CCT GGT CGT CGA CAC GGC CCT GGC CTC CGC GGC CCT CGG CGC CGG ATG Arg Ala Gly Pro Gly Arg Arg His Gly Pro Gly Leu Arg Gly Pro Arg Arg Arg Met					
	3070	3080	3090	3100	3110	3120
15	ACC GGC GGC GGC TTC GGC GGC TCG GCG ATC GTC CTG GTG GAG GCC GCC GCG GTG GAC Thr Gly Gly Gly Phe Gly Gly Ser Ala Ile Val Leu Val Glu Ala Ala Ala Val Asp					
	3130	3140	3150	3160	3170	3180
	GCC GTC ACC AAG GCG GTC GAG GAC GCC TTC GCC GCG GCG GGC CTC AAG CGT CCG CGG Ala Val Thr Lys Ala Val Glu Asp Ala Phe Ala Ala Ala Gly Leu Lys Arg Pro Arg					
20	3190	3200	3210	3220	3230	3240
	GTG TTC GAG GCG GTG CCT CGG CCG GGC GCG GCG CCT GGT CTG ACG GTC AGC CGA GCC Val Phe Glu Ala Val Pro Arg Arg Gly Ala Ala Pro Gly Leu Thr Val Ser Arg Ala					
	3250	3260	3270	3280	3290	
25	GCT TCA CCA GCG TGT ACT CCG TGA TCC CCG GCG GGT AGT CCG GGA TCA CGC ACA TGA Ala Ser Pro Ala Cys Thr Pro ---					
	3300					
	GCT GCT AGC CGC					
30						
35						

1

EXAMPLE 2

PROMOTERS OF THE S. LIVIDANS GAL OPERON

a) P1 promoter

(i) Summary

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This promoter is galactose inducible, glucose repressible and is the regulatable promoter for the entire Streptomyces gal operon. S1 data indicates that the Streptomyces lividans gal operon encodes a polycistronic transcript of approximately 3.4 kilobases (Kb). The transcript consists of approximately 1 Kb for galT, followed by approximately 1 Kb each for galE and galK. (See, Figure 1).

15

Galactose induction of P1 is mediated, at least in part, by an operator sequence whose 5' end is located 31 bp upstream of the transcription start site and a repressor protein which recognizes the operator.

(ii) Experimental: Isolation, Localization, and Characterization of the P1 promoter.

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The sequences upstream of the Streptomyces lividans galK ATG were screened for promoters using the E. coli galK promoter probe system of Brawner, et al., Gene, 40, 191, (1985), in press. The HindIII-MluI fragment (See, Table A, map positions 1-5) was restricted with Sau3AI, ligated into the unique BamHI site of pK21 (Figure 2), and transformed into E. coli K21 (galK⁻) according to the method of Example 1. pK21 is a derivative of pSK03 and is an E. coli-Streptomyces shuttle vector containing the E. coli galK gene (See, Figure 2). The construction of pSK03 is described in Rosenberg et al., Genetic Engineering, 8, (1986), in press. The clones which expressed galK, i.e., those which had promoter activity, were identified on MacConkey - galactose plates. Two galK⁺ clones (designated as pK21 MH1 and 2) were transformed into Streptomyces 1326-12K (galK⁻).

1

Extracts from transformants were cultured in Ymglu and Ymgal, and were analyzed by western blot analysis using anti-E. coli galactokinase antiserum. The blots showed significantly higher levels of galactokinase in the extracts from the galactose induced cultures.

5

pK21 MH1 and 2 were shown by restriction analysis to contain a 410 bp Sau3AI insert which is contained within the HindIII and BglII sites (see Table A, map positions 1-2) by Southern blot analysis according to the method of Southern, J. Mol. Biol., 98, 503 (1975). The cloned fragment was analyzed by S1 analysis using RNA isolated from Streptomyces lividans 1326-12K and E. coli K21 cultures. The fragment yielded a 290 nucleotide protected fragment after S1 digestion (indicating the 5' end of an mRNA 290 bp upstream of the Sau3AI site). Hybridization experiments (using single stranded M13 clones of this region) have identified the direction of transcription as left to right as shown in Figure 2 (i.e., transcription is going toward galK).

10

15

20

Conventional DNA sequence analysis and additional S1 mapping analysis were used to define the 5' end of the mRNA.

The sequences responsible for regulating galactose induction of P1 were localized by removing sequences upstream of the transcription start site by nuclease Bal31. Any change in promoter function or galactose induction by removal of these sequences was assessed using the E. coli galK promoter probe plasmid used to identify P1.

25

30

(iii) Construction of Gal Promoter Deletions.

Plasmid pHL5 was constructed by cloning a DNA fragment containing 100 bp of sequences downstream from the start of P1 transcription and 216 bp upstream from the start of P1 transcription into plasmid pUC19TT1. Plasmid pUC19TT1 is described in Norrander et al., Gene, 26, 101-106 (1983) and has the Unker as pUC18-TT6. See, Example IB. Deletions extending into the upstream

35

1 sequence preceeding P1 were generated by linearizing pHL5
with HindIII and treating the ends with nuclease Bal31.
The uneven ends were subsequently repaired with the Klenow
fragment of DNA polymerase I. Bal31-treated pHL5 was then
5 digested with BamHI and run on a 5% acrylamide gel. DNA
fragments in the molecular weight range of 100-300 bp were
eluted from the gel and subcloned into M13 mp 10 that had
been digested with HindII and BamHI. [See, Messing,
Methods in Enzymology, 101, 20 (1983)]. Individual
10 deletions were then sequenced from the single stranded
phage DNA the dideoxy chain termination method of Sanger,
et al., cited above.

(iv) Linking the P1 Promoter Deletions to the
E. coli galK Gene.

15 The various mp 10 clones were digested with BamHI
and HindIII. DNA fragments containing individual
deletions were isolated from low-melting point agarose
gels and then ligated to pK21 (see, Figure 2) that had
been digested with BamHI and HindIII. After
20 transformation into E. coli MM294, plasmid DNA was
isolated for each of the deletion derivatives and
transformed into Streptomyces lividans 12K.

(v) Functional Assessment of Bal
31-Generated Deletions in S. lividans

25 For each individual promoter deletion, a single
thiostrepton resistant transformant was grown to late log
in YM base (YEME) + 10 ug/ml thiostrepton. Cells were
then pelleted, washed once in M56 media and resuspended in
M56 media (see Miller, et al., cited above). The washed
30 cells were then used to inoculate YM + 0.1M MOPS (pH 7.2)
+ 10 ug/ml thiostrepton supplemented with 1% galactose or
1% glucose. The cells were grown for 16 hours then
assayed for galactokinase activity.

Ten individual pK21 derivatives containing either
35 120, 67, 55, 34, 31, 24, 20, 18, 10 or 8 bp of sequence
upstream of the P1 transcription start site were analyzed

1

for galactokinase expression. These results showed that all the information necessary for galactose induction of P1, (i.e., 10-20 fold greater levels of galactokinase produced in galactose grown cells versus glucose grown cells) is included in the 31 bp of sequence upstream of P1. A deletion which leaves 34 bp of sequence upstream of P1 is partially inducible by galactose since galactose induced 6-fold greater amounts of galactokinase. Thus, one end of the operator must be situated within the sequences between the -24 and -31 position. The remaining deletions which leave either 20, 18, 10 or 8 bp of upstream sequence result in a constitutive P1 promoter, that is the levels of galactokinase produced were equivalent when cells were grown in the presence of galactose or glucose. Although the promoter deletions which retained 8 and 10 bp of P1 were constitutive, the amount of galactokinase produced was reduced 10 fold in comparison to the promoter deletions which retained 18 to 120 bp of upstream sequence. This result indicates that sequences between the -10 and -18 positions of -1 are essential for promoter function.

This data supports a model in which galactose induction of P1 is mediated, at least in part, by an operator sequence. One end of this sequence is 24 to 31 bp upstream of the P1 transcription start site. Removing part or all of the operator results in a promoter which is partially or totally derepressed. The other end of this sequence has not been defined by these experiments but it most likely is contained within the 24 to 31 bp of sequence upstream of the P1 transcription start site. In addition we cannot eliminate the possibility that the 3' end of the operator is also within the 100 bp downstream of the transcription start site since these sequences were contained within the smallest region needed to achieve galactose induction. These data also suggest that the factor which interacts with the operator sequence is a

1 repressor protein. Finally, we do not have any evidence
which eliminates the possibility that P1 may be controlled
by factors other than a repressor (i.e., positive
5 activator such as lambda phage cII protein) to modulate
galactose induction promoter transcript.

b) P2 promoter

(i) Summary

The P2 promoter of the Streptomyces gal
10 operon is upstream of the galE gene and transcribes both
galE and galK genes.

P2 promoter expression is constitutive
(i.e., not glucose repressed/galactose induced) as shown
by S1 analysis.

15 (ii) Experimental: Isolation, Localization,
and Characterization of the P2 promoter.

The existence of the Streptomyces gal operon P2
promoter became apparent when the BglII-MluI fragment
(see, Table A, map positions 2-5) of S. lividans 1326 DNA
20 was inserted into plasmid pK21 (see, Figure 2) and
galactokinase expression was observed in Streptomyces
lividans 1326-12K transformed therewith.

DNA sequence analysis and S1 analysis were used
to identify the 5' end of the S. lividans gal operon P2.
25 The 5' end of the P2 promoter transcript is within 100 bp
upstream of the predicted galE ATG.

EXAMPLE 3

EVIDENCE OF A POLYCISTRONIC MESSAGE IN THE
30 STREPTOMYCES GAL OPERON

S1 analysis was used to map the transcripts
upstream and downstream of the Streptomyces lividans gal
operon galK gene. In general, overlapping DNA fragments
35 of 1-2 Kb were isolated from subclones, further
restricted, and end labelled. The message was followed
from the 3' end of galK to the upstream end at P1.

1

The 3' end of the Streptomyces lividans gal operon transcript probably occurs within the first hundred bases downstream of galK. Fragments 3' labelled at sites
5 within the galK sequence were not protected to their full length (S1 analysis) if they extend into this downstream region. One experiment showed a possible protected region that terminated 50-100 bp downstream of the galK translation stop. The existence of a transcription
10 terminator can be confirmed by conventional techniques by using a terminator probe system. The gal operon transcript clearly does not extend to the PvuII site (see, Table A, map position 8) because no full length protection of 5' labelled PvuII fragments occurs from that site.

15

5' end labelled fragments from two PvuII fragments, fragment I, (map positions 4-6, See, Table A), and fragment II, (map positions 6-8, See Table A), and the insert of pSaul0 were used as sources of probes for S1 walking from the 3' to 5' end of the message. All
20 fragments through this region are protected, except the fragment containing the P2 promoter which shows partial and full protection. The complete protection from S1 digest indicates a polycistronic message which initiates upstream at P1 and continues to approximately 100 bp
25 downstream of galK.

30

The above data is indirect evidence of a polycistronic mRNA of the Streptomyces gal operon. S1 analysis using a long contiguous DNA fragment (e.g., the 4.5 kb HindIII-SacI fragment, see map position 7 of Table
35 A) has been used to confirm the transcript size.

35

1

EXAMPLE 4

LOCALIZATION OF S. LIVIDANS GAL OPERON galE AND galT GENES

5 (i) Summary

The S. lividans gal operon galE gene was localized to 1.5 Kb PvuII fragment (map position, 4-6 of Table A) of pLIVGAL1 (Figure 1).

10 The S. lividans gal operon galE coding sequences extend through the MluI site (map position 5 of Table A).

The S. lividans gal operon galT gene was localized within the 1.15 Kb Nru-PvuII region (see, Table A, map positions 1a-4) of pSLIVGAL1.

15 The direction of S. lividans gal operon galE and galT transcription is the same as galK gene.

(ii) Experimental

It was necessary to identify the other functions contained on pLIVGAL1; specifically, does this plasmid encode for the enzyme galactose epimerase (galE) or the enzyme galactose transferase (galT). The Streptomyces gal operon galK gene was identified by its ability to complement an E. coli galK host. Thus, identification of the Streptomyces galT and galE genes was tested for by complementation of E. coli galE⁻ or galT⁻ hosts, respectively. An E. coli galT⁻ strain (CGSC strain #4467, W3101) and two galE⁻ strains (CGSC strain #4473; W3109 and CGSC strain #4498; PL-2) were obtained to test for complementation by the pSLIVGAL1 clone.

30 The ca. 9 Kb HindIII-SphI fragment (see, Table A, map positions 1-16) containing the Streptomyces lividans gal operon galK gene was inserted into pUC19. This fragment was situated within pUC19 such that transcription from the Plac promoter of pUC19 is in the same direction as the Streptomyces galK gene. pUC19 is described in Yanisch-Perrou, et al., Gene, 33, 103 (1983).
35 Complementation was assayed by growth on MacConkey-galactose plates. Cells which can utilize galactose

1 [galE⁺, galT⁺, galK⁺] will be red to pink on this
medium. E. coli strain PL-2 (see, Example 2) containing
pUC19 with the HindIII-SphI insert were pink on the
5 indicator plate indicating that the HindIII-SphI fragment
contains the Streptomyces lividans galE gene. The galE
gene was later mapped to within the 4.5 Kb HindIII-SacI
(the SacI site is near the region around map position 7-8
of Table A) fragment. If the sequences from the MluI site
10 (map position 5 of Table A) to the SacI site were removed
galE complementation of E. coli PL-2 was not detected.
The 5' end of the galK gene is 70 base pairs (bp) from the
MluI site. Therefore it seemed likely that the MluI site
was contained within the 5' or 3' end of the galE gene.
15 To determine the direction of galE transcription, the
HindIII-SacI fragment was inserted into pUC18. In this
configuration, the Streptomyces lividans galK gene is in
the opposite orientation with respect to Plac. The pUC18
HindIII-SphI clone did not complement E. coli PL-2
20 indicating the galE is transcribed in the same direction
as galK. In addition it was concluded that the MluI site
is contained within the 3' end of the galE gene. DNA
sequence analysis of the PvuII-MluI fragment (See, Table
A, map position 4-5) has identified an open reading frame
25 which encodes for a polypeptide of predicted molecular
weight of 33,000 daltons. The 5' end of this reading
frame is located approximately 176 bp from the PvuII site
(See, Table A, map position 4). Therefore, the sequencing
results support the conclusion that the 3' end of galE
30 traverses the MluI site (see, Table A, map position 5).

Similar experiments to localize the galT gene on
pSLIVGAL1 were attempted with the galT hosts.

The region between P1 and the 5' end of galE was
sequenced to identify the galT gene. Translation of the
DNA sequence to the amino acid sequence identified a
35 reading frame which encodes a protein showing a region of
homology to the yeast transferase.

1

EXAMPLE 5

GALACTOSE INDUCTION OF S. LIVIDANS GAL OPERON GALK GENE

5

(i) Summary

Galactokinase expression is induced within one hour after the addition of galactose to culture medium.

10 Galactokinase expression is 10 times higher in the presence of galactose versus glucose or no additional carbon source within 6 hours after addition of the sugar.

(ii) Experimental

15 Galactose induction of the Streptomyces lividans galK gene was examined by assaying for galactokinase activity at 1, 3, 6 and 24 hours after the addition of galactose. Two liters of YM + 0.1M MOPS (pH 7.2) were inoculated with 2×10^7 spores of Streptomyces lividans 1326. After 21 hours growth, galactose or glucose were added to a final concentration of 1%. One, three, six and 20 twenty four hours after the addition of sugar, cells were isolated and assayed for galactokinase activity. Total RNA was prepared by procedures described in Hopwood et al., cited above.

25 An increase in galactokinase synthesis was observed one hour after the addition of galactose. The increase continued over time (1 to 24 hours). S1 analysis of RNA isolated from the induced cultures confirmed that the increase in galK activity was due to increased levels of the P1 promoter transcript.

30 The S1 data and the induction studies suggest the following model for gene expression within the Streptomyces gal operon. The P1 promoter is the galactose inducible promoter. The P1 transcript includes galT, galE and galK. The P2 promoter is constitutive and its 35 transcript includes galE and galK.

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It is interesting to note that the E. coli gal operon also has two promoters, P1 and P2. [See, Nusso et al., Cell, 12, 847 (1977)]. P1 is activated by cAMP-CRP binding whereas P2 is inhibited by cAMP-CRP. Translation of the E. coli gal operon galE coding sequence is more efficient when transcription initiates at P2 which serves to supply a constant source of epimerase even in the absence of galactose or the presence of glucose [See, Queen et al., Cell, 25, 241 (1981)]. The epimerase functions to convert galactose to glucose 1-phosphate during galactose utilization and convert UDP-glucose to UDP-galactose which is required for E. coli cell wall biosynthesis. It is possible that the P2 promoter of the Streptomyces galK operon also serves to supply epimerase and galactokinase in the absence of galactose or during secondary metabolism.

20

EXAMPLE 6
THE S. COELICOLOR GAL OPERON

(i) Summary

The restriction map of a fragment containing the S. coelicolor galK gene is identical to the restriction map of the S. lividans gal operon. (See, Figure 3).

S. coelicolor can grow on minimal media containing galactose as the sole carbon source.

Galactokinase expression in S. coelicolor is induced by the addition of galactose to the growth media.

A promoter analogous and most likely identical to P1 is responsible for galactose induction of the S. coelicolor gal operon.

(ii) Experimental

An approximately 14 kb partial Sau3A fragment containing the S. coelicolor galK gene was isolated by K. Kendall and J. Cullum at the University of Manchester Institute of Science and Technology, Manchester, UK

1 (unpublished data; personal communication). They were
able to localize the S. coelicolor galK gene within a 3 kb
EcoRI fragment by complementation of a S. coelicolor galK
mutant. The position of a number of restriction sites
5 within the S. lividans gal operon are identical to those
found within, upstream and downstream of the EcoRI
fragment containing the S. coelicolor galK gene
(Figure 3). Thus, it seems likely that the gene
organization of the S. coelicolor gal operon is identical
10 to the S. lividans gal operon.

Galactose induction of the S. coelicolor galK
gene was examined by immunoblotting. S. coelicolor was
grown in Ym + 1% galactose or 1% glucose (Ymglu or Ymgal)
for 20 hours at 28 C. Galactokinase expression was
15 detected using rabbit antisera prepared against purified
E. coli galactokinase. The protein detected was the
approximate size of the E. coli and S. lividans galK gene
product. Galactokinase expression is galactose induced
since it was detected only when S. coelicolor was grown in
20 Ym + galactose (Ymgal).

S1 nuclease protection studies were performed to
determine if galactose induction of the S. coelicolor gal
operon is directed by a promoter analogous to the S.
lividans P1 promoter. RNA was isolated from S. coelicolor
25 grown in Ym + 1% galactose or 1% glucose (Ymgal or
Ymglu). The hybridization probe used for S1 analysis of
this RNA was a 410 bp Sau3A fragment which contains the S.
lividans P1 promoter, its transcription start site and the
5' end of the galT gene. The S1 protected fragment
30 detected by this analysis co-migrated with the protected
fragment detected when the probe was hybridized to RNA
isolated from S. lividans grown in the presence of
galactose. Thus, this result shows that galactose
induction of the S. coelicolor gal operon is directed by a
35 sequence indistinguishable from the S. lividans P1
promoter.

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It should be noted that the following strains of Streptomyces have been observed to be able to grow on medium containing galactose as the only carbon source:

5

S. albus J1074 (obtained from Dr. Chater, John Innes Foundation, Norwich, England)

S. carzinostaticus - ATCC accession number 15944

S. carzinostaticus - ATCC accession number 15945

10 S. antifibrinolyticus - ATCC accession number 21869

S. antifibrinolyticus - ATCC accession number 21870

S. antifibrinolyticus - ATCC accession number 21871

S. longisporus - ATCC accession number 23931

15

The abbreviation "ATCC" stands for the American Type Culture Collection, Rockville, Maryland, U.S.A.

20

While the above descriptions and Examples fully describe the invention and the preferred embodiments thereof, it is understood that the invention is not limited to the particular disclosed embodiments. Thus, the invention includes all embodiments coming within the scope of the following claims.

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Claims for the Contracting States :
BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

5

1. A recombinant DNA molecule comprising a Streptomyces gal operon or any regulatable and functional derivative thereof.

10

2. The molecule of Claim 1 wherein the operon is a S. lividans, S. coelicolor, S. azuraeus, S. albus, S. carzinostaticus, S. antifibrinolyticus or S. longisporus gal operon.

15

3. The molecule of Claim 2 wherein the operon is a S. lividans gal operon.

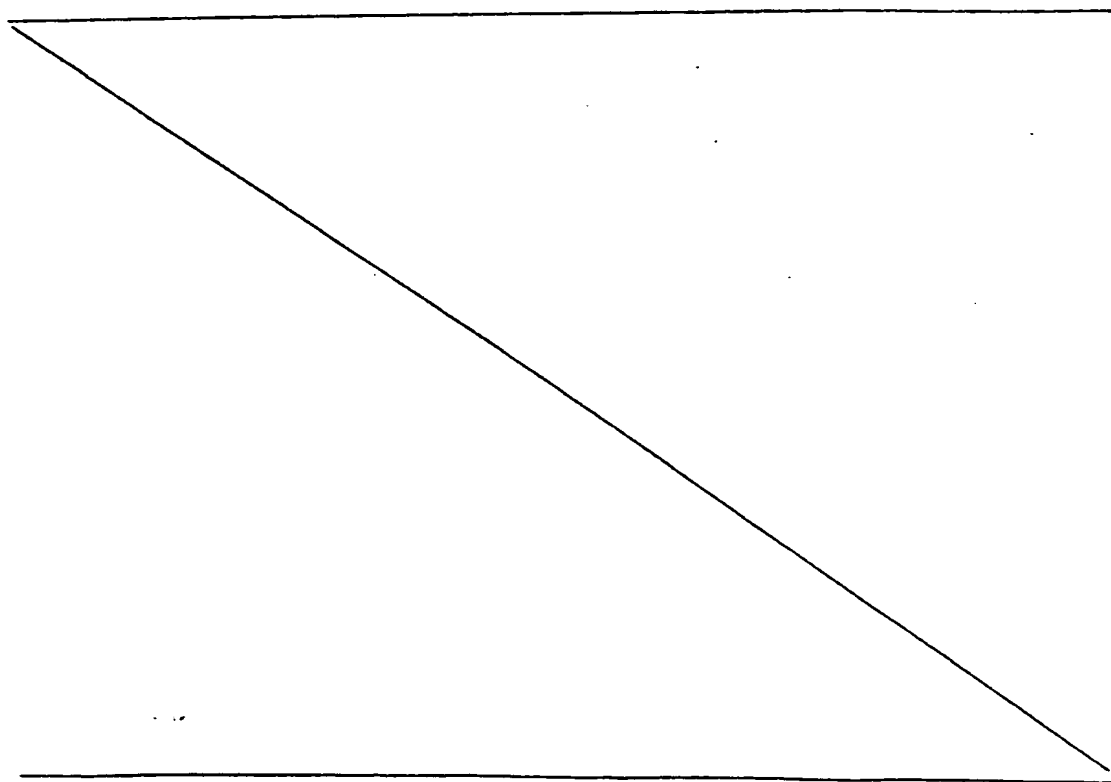
4. The molecule of Claim 3 which has the following coding sequence:

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340 350 360 370 380
 GGT GAC TCC GCG CGC TGC GAG GTC CTC TGC TTC ACC TCC GAC CAC GAC GCC TCC TTC
 Gly Asp Ser Gly Arg Cys Glu Val Val Cys Phe Thr Ser Asp His Asp Ala Ser Phe
 5
 390 400 410 420 430 440
 GCC GAC CTG ACC GAG GAG CAG GCC CGG CTG GTC GTC GAC GCC TGG ACC GAC CGC ACC
 Ala Asp Leu Ser Glu Glu Gln Ala Arg Leu Val Val Asp Ala Trp Thr Asp Arg Thr
 10
 450 460 470 480 490 500
 TCC GAG CTG TCC CAT CTG CCC TCC GTT CAA CAG GTC TTC TGC TTC GAG AAC CGG GCG
 Ser Glu Leu Ser His Leu Pro Ser Val Glu Gln Val Phe Cys Phe Glu Asn Arg Gly
 15
 510 520 530 540 550
 GCC GAG ATC GGG GTG ACC CTG GGT CAC CCG CAC GGG CAG ATC TAC GCC TAC CCG TTC
Ala Glu Ile Gly Val Thr Leu Gly His Pro His Gly Gln Ile Tyr Ala Tyr Pro Phe
 560 570 580 590 600 610
 ACC ACC CCC CGC ACC GCC CTG ATG CTC CGT TCA CTC GCC GCC CAC AAG GAC GCG ACC
 Thr Thr Pro Arg Thr Ala Leu Met Leu Arg Ser Leu Ala Ala His Lys Asp Ala Thr
 20
 620 630 640 650 660 670
 GGC GGG GGG AAC CTG TTC GAC TCC GTG CTG GAG GAG GAG CTG GCC GGT GAG CGG GTC
 Gly Gly Gly Asn Leu Phe Asp Ser Val Leu Glu Glu Glu Leu Ala Gly Glu Arg Val
 25
 680 690 700 710 720
 GTC CTG GAG GGT GAG CAC TGG GCC GCC TTC GTC GCG TAC GGC GCG CAC TGG CCG TAC
 Val Leu Glu Gly Glu His Trp Ala Ala Phe Val Ala Tyr Gly Ala His Trp Pro Tyr
 730 740 750 760 770 780
 GAG GTG CAC CTC TAC CCG AAG CGG CGG GTG CCC GAT CTG CTC GGG CTC GAC GAG GCG
 Glu Val His Leu Tyr Pro Lys Arg Arg Val Pro Asp Leu Leu Gly Leu Asp Glu Ala
 30
 790 800 810 820 830 840
 GCT CGC ACA GAA TTC CCC AAG GTC TAC CTG GAG CTG CTG AGG CGT TTC GAC CGG ATC
 Ala Arg Thr Glu Phe Pro Lys Val Tyr Leu Glu Leu Leu Arg Arg Phe Asp Arg Ile
 35

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5 850 860 870 880 890 900
 TTC GGC GAG GGC GAG CCC CCC ACC CCC TAC ATC GCG GCC TCG CAC CAG CCG CCG TTC
 Phe Gly Glu Gly Glu Pro Pro Thr Pro Tyr Ile Ala Ala Trp His Gln Ala Pro Phe
 910 920 930 940 950
 GCG CAC CTG CAG TTC GAG GGT GTC ACC CCG GAC GAC TTC GCC CTC CAC CTG GAA CTT
 Gly Gln Leu Glu Phe Glu Gly Val Thr Arg Asp Asp Phe Ala Leu His Leu Glu Leu
 10 960 970 980 990 1000 1010
 TTC ACT TCC GCC GTA CGT CCG GCA AGC TGA AGT TCC TCG CCG GCT CCG AAT CCG GCA
 Phe Thr Ser Ala Val Arg Pro Ala Ser --- galP2
 1020 1030 1040 1050 1060 1070
 15 TGAACG TGTTTCATCAA CGACGTACCC CCGGAGCGCG CCGCCGAGCC ACTGCGAGAG GTAGCGAG
 1080 1090 1100 1110 1120 1130
 TTC ATG AGC GGG AAG TAC CTG GTG ACA GGT GGT GCC GGA TAC GTC GGC AGC GTC GTC
 Met Ser Gly Lys Tyr Leu Val Thr Gly Gly Ala Gly Tyr Val Gly Ser Val Val
 20 1140 1150 1160 1170 1180 1190
 GCG CAG CAC TTG GTG GAG GCG GGG AAC GAG GTC GTG GTG CTG CAC AAT CTG TCG ACC
 Ala Gln His Leu Val Glu Ala Gly Asn Glu Val Val Val Leu His Asn Leu Ser Thr
 1200 1210 1220 1230 1240
 25 GGC TTC CGT GAG GTG TCG CCG CCG GTC CCT CGT TCG TCG AGG CGA CAT CCG GGA CCG
 Gly Phe Arg Glu Val Cys Arg Arg Val Pro Arg Ser Ser Arg Arg His Pro Gly Arg
 1250 1260 1270 1280 1290 1300
 CGC CAA GTG CGT GGA CCG CTC TCG TTC GAC GGC GTG CTG CAC TTC GCC GCC TTC TCC
 Arg Gln Val Arg Gly Arg Leu Ser Phe Asp Gly Val Leu His Phe Ala Ala Phe Ser
 30 1310 1320 1330 1340 1350 1360
 CAG GTC GGC GAG TCG GTC GTG AAG CCC GAG AAG TAC TCG GAC AAC AAC GTC GGT GGC
 Gln Val Gly Glu Ser Val Val Lys Pro Glu Lys Tyr Trp Asp Asn Asn Val Gly Gly

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1370 1380 1390 1400 1410 1420
 5 ACC ATG GCC CTG CTC GAG GCC ATG CGG GCC GCC GGT GTG CGG CGG CTC GTC TTC TCC
 Thr Met Ala Leu Leu Glu Ala Met Arg Gly Ala Gly Val Arg Arg Leu Val Phe Ser
 1430 1440 1450 1460 1470
 TCC ACG GCC GCC ACC TAC GCC GAG CCC GAG CAG GTT CCC ATC GTC GAG TCC GCC CCG
 Ser Thr Ala Ala Thr Tyr Gly Glu Pro Glu Gln Val Pro Ile Val Glu Ser Ala Pro
 10 1480 1490 1500 1510 1520 1530
 ACG AGG CCC ACC AAT CCG TAC GCC GCC TCG AAG CTC GCC GTC GAG CAC ATC ATC ACC
 Thr Arg Pro Thr Asn Pro Tyr Gly Ala Ser Lys Leu Ala Val Asp His Met Ile Thr
 1540 1550 1560 1570 1580 1590
 15 GGC GAG CCG GCG GCC CAC GGG CTG GGC GCG GTC TCC GTG CCG TAC TTC AAC GTC GCG
 Gly Glu Ala Ala Ala His Gly Leu Gly Ala Val Ser Val Pro Tyr Phe Asn Val Ala
 1600 1610 1620 1630 1640
 GGC GCG TAC GGG GAG TAC GCC GAG CGC CAC GAG CCC GAG TCG CAT CTG ATT CCC CTG
 Gly Ala Tyr Gly Glu Tyr Gly Glu Arg His Asp Pro Glu Ser His Leu Ile Pro Leu
 20 1650 1660 1670 1680 1690 1700
 GTC CTT CAA GTG GCG CAG GGC AGG CCG GAG GCC ATC TCC GTC TAC GGC GAG GAG TAC
 Val Leu Gln Val Ala Gln Gly Arg Arg Glu Ala Ile Ser Val Tyr Gly Asp Asp Tyr
 1710 1720 1730 1740 1750 1760
 25 CCG ACC CCG GAC CGA CCT GTG TGC GCG ACT ACA TCC ACC TCG CCG ACC TCG CCG AGG
 Pro Thr Pro Asp Arg Pro Val Cys Ala Thr Thr Ser Thr Ser Pro Thr Trp Pro Arg
 1770 1780 1790 1800 1810
 CCC ACC TGC TGG CCG TGC GCC GCC GCC CCG GGC GAG CAC CTC ATC TGC AAC CTG GGC
 Pro Thr Cys Trp Pro Cys Ala Ala Ala Pro Gly Glu His Leu Ile Cys Asn Leu Gly
 30 1820 1830 1840 1850 1860 1870
 AAC GGC AAC GGC TTC TCC GTC CGC GAG GTC GTC GAG ACC GTG CCG CCG GTG ACC GGC
 Asn Gly Asn Gly Phe Ser Val Arg Glu Val Val Glu Thr Val Arg Arg Val Thr Gly

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-60-

2390 2400 2410 2420 2430 2440
 5
 CGC GAG GCC GGA CAC GAG CTG ACC GGC GCC GAC GTC CAC CTG GCC TCG ACC GTC CCG
 Arg Glu Ala Gly His Glu Leu Thr Gly Ala Asp Val His Leu Ala Ser Thr Val Pro
 2450 2460 2470 2480 2490
 10
 TCC GCG GCG GCG CTC TCC TCC TCC GCG GCC CTG GAG GTC CGT CCC CTG GCG ATG AAC
 Ser Gly Ala Gly Leu Ser Ser Ser Ala Ala Leu Glu Val Arg Pro Leu Ala Met Asn
 2500 2510 2520 2530 2540 2550
 15
 GAC CTG TAC GCC CTC GCG CTG CCG GCG TGG CAG CTG GCC CCG CTG TCC CAG CCG GCG
 Asp Leu Tyr Ala Leu Ala Leu Arg Gly Trp Gln Leu Ala Arg Leu Cys Gln Arg Ala
 2560 2570 2580 2590 2600 2610
 20
 GAG AAC GTC TAC GTC GCG GCC CCC GTC GCG ATC ATC GAC CAG ACC GCG TCC GCC TGC
 Glu Asn Val Tyr Val Gly Ala Pro Val Gly Ile Met Asp Gln Thr Ala Ser Ala Cys
 2620 2630 2640 2650 2660 2670
 25
 TGC GAG GCG GCG ACG CCC TCT TCC TCG ACA CCC GCG ACC TCT CCC ACC GCG ACA TCC
 Cys Glu Ala Gly Thr Pro Ser Ser Ser Thr Pro Ala Thr Ser Pro Ser Gly Arg Ser
 2680 2690 2700 2710 2720
 30
 CCT TCG ACC TCG CCG CCG AGG GGA TGC GCC TGC TGG TCG TCG ACA CCC GCG TCA AGC
 Pro Ser Thr Ser Pro Pro Arg Gly Cys Ala Cys Trp Ser Ser Thr Pro Gly Ser Ser
 2730 2740 2750 2760 2770 2780
 35
 ACT CCC ACA GCG AGG GCG AGT ACG GCA AGC GCC GCG CCG GCT GCG ACA AGG GCG CCG
 Thr Pro Thr Ala Arg Ala Ser Thr Ala Ser Ala Ala Arg Ala Ala Arg Arg Ala Pro
 2790 2800 2810 2820 2830 2840
 40
 CGC TGC TGG GCG TCG ACG CCG TGC GAC GTG CCG TAC GCC GAC CTG GAC GCG CCG CTG
 Arg Cys Trp Ala Ser Thr Arg Cys Asp Val Pro Tyr Ala Asp Leu Asp Ala Ala Leu
 2850 2860 2870 2880 2890
 45
 GAG CCG CTG GCG GAC GAG GAG GAG GTG CCG CCG CTG GTC CCG CAC GTG GTC ACC GAG
 Glu Arg Leu Gly Asp Glu Glu Glu Val Arg Arg Leu Val Arg His Val Val Thr Glu

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2900 2910 2920 2930 2940 2950
 5 GAC GAG CGC CTC GAA CGC CTC GTC GCC CTC CTC GAG TCG CGC ACA CCC GGC GCA TCG
 Asp Glu Arg Val Glu Arg Val Val Ala Leu Leu Glu Ser Ala Thr Pro Gly Ala Ser
 2960 2970 2980 2990 3000 3010
 GCG CCG TCC TCG TCG AGG GCC ACC CCT GCT GCG CGA CGA CTT CCG CAT CTC CTC CCC
 Ala Pro Ser Trp Ser Arg Ala Thr Pro Ala Ala Arg Arg Leu Pro His Leu Leu Pro
 10 3020 3030 3040 3050 3060
 CGA GCT GGA CCT GGT CGT CGA CAC GGC CCT GGC CTC CGC GGC CCT CCG CGC CGG ATG
 Arg Ala Gly Pro Gly Arg Arg His Gly Pro Gly Leu Arg Gly Pro Arg Arg Arg Met
 3070 3080 3090 3100 3110 3120
 15 ACC GGC GGC GGC TTC GGC GGC TCG GCG ATC GTC CTC GTG GAG GCC GCC GCG GTG GAC
 Thr Gly Gly Gly Phe Gly Gly Ser Ala Ile Val Leu Val Glu Ala Ala Ala Val Asp
 3130 3140 3150 3160 3170 3180
 GCC GTC ACC AAG GCG GTC GAG GAC GCC TTC GCG GCG GCG GCG CTC AAG CGT CCG CGG
 Ala Val Thr Lys Ala Val Glu Asp Ala Phe Ala Ala Ala Gly Leu Lys Arg Pro Arg
 20 3190 3200 3210 3220 3230 3240
 GTC TTC GAG GCG GTG CCT CGG CGG GCG GCG GCG CCT GCT CTC ACC GTC AGC CGA GCC
 Val Phe Glu Ala Val Pro Arg Arg Gly Ala Ala Pro Gly Leu Thr Val Ser Arg Ala
 3250 3260 3270 3280 3290
 25 GCT TCA CCA GCG TGT ACT CCG TGA TCC CCG GCG GGT ACT CCG GGA TCA CGC ACA TGA
 Ala Ser Pro Ala Cys Thr Pro ---
 3300
 GCT GCT AGC CGC

30

35

- 1 5. The molecule of Claim 1 which further
comprises a foreign functional DNA sequence operatively
linked to such operon.
- 5 6. A transformed host microorganism or cell
comprising the molecule of Claim 5.
7. A method of preparing a transformed host
microorganism or cell comprising the molecule of Claim 5
which comprises transforming an appropriate host
microorganism or cell with such molecule.
- 10 8. A recombinant DNA vector comprising the
molecule of Claim 5, and, optionally, additionally
comprising a replicon.
9. A transformed host microorganism or cell
comprising the recombinant DNA vector of Claim 8.
- 15 10. A method of preparing a transformed host
microorganism or cell comprising the recombinant DNA
vector of Claim 8 which comprises transforming an
appropriate host microorganism or cell with such vector.
11. A method of expressing a foreign functional
20 DNA sequence which comprises cultivating a transformed
host microorganism or cell comprising the recombinant DNA
vector of Claim 8 under suitable conditions such that the
functional DNA sequence is expressed.
12. A method of regulating the expression of a
25 foreign functional DNA sequence which comprises
cultivating a transformed host microorganism or cell which
contains the recombinant DNA vector of Claim 8 under
appropriate conditions such that expression of the
sequence is regulatable.
- 30 13. A recombinant DNA molecule comprising a
Streptomyces gal operon P2 promoter expression unit or any
functional derivative thereof.
14. The molecule of Claim 13 wherein the
expression unit is a S. lividans, S. coelicolor, S.
35 azuraeus, S. albus, S. carzinostaticus, S.
antifibrinolyticus or S. longisporus gal operon P2
promoter expression unit.

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11. The molecule of Claim 14 which is a S. lividans gal operon P2 promoter expression unit.

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16. The molecule of Claim 13 which further comprises a foreign functional DNA sequence operatively linked to such expression unit.

17. A transformed host microorganism or cell comprising a recombinant DNA molecule wherein such molecule comprises the molecule of Claim 16.

10

18. A method of preparing a transformed host microorganism comprising the molecule of Claim 16 which comprises transforming an appropriate host microorganism or cell with such molecule.

15

19. A recombinant DNA vector comprising the molecule of Claim 16, and, optionally, additionally comprising a replicon.

20. A transformed host microorganism or cell comprising the recombinant DNA vector of Claim 19.

20

21. A method of preparing a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 19 which comprises transforming an appropriate host microorganism with such vector.

25

22. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 19 under suitable conditions such that the functional DNA sequence is expressed.

23. A recombinant DNA molecule comprising a Streptomyces gal operon P1 promoter regulated region or any regulatable and functional derivative thereof.

30

24. The molecule of Claim 23 wherein the region is a S. lividans, S. coelicolor, S. azuraeus, S. albus, S. carzinostaticus, S. antifibrinolyticus or S. longisporus gal operon P1 promoter regulated region.

35

25. The molecule of Claim 24 wherein the region is a S. lividans gal operon P1 promoter regulated region.

1 26. The molecule of Claim 23 which further
comprises a foreign functional DNA sequence operatively
linked to such regulated region.

5 27. A transformed host microorganism or cell
comprising the molecule of Claim 26.

 28. A method of preparing a transformed host
microorganism or cell comprising the molecule of Claim 26
which comprises transforming an appropriate host
microorganism or cell with such molecule.

10 29. A recombinant DNA vector comprising the
molecule Claim 26, and, optionally, additionally
comprising a replicon.

 30. A transformed host microorganism or cell
comprising a recombinant DNA vector of Claim 29.

15 31. A method of preparing a transformed host
microorganism or cell comprising the recombinant DNA
vector of Claim 29 which comprises transforming an
appropriate host microorganism or cell with such vector.

20 32. A method of expressing a foreign functional
DNA sequence which comprises cultivating a transformed
host microorganism or cell comprising the recombinant DNA
vector of Claim 29 under suitable conditions such that the
functional DNA sequence is expressed.

25 33. A method of regulating the expression of a
foreign functional DNA sequence which comprises
cultivating a transformed host microorganism or cell which
contains the recombinant DNA vector of Claim 29 under
appropriate conditions such that expression of the
sequence is regulatable.

30 34. A recombinant DNA molecule comprising a
Streptomyces gal operon P2 promoter or any functional
derivative thereof.

35 35. The molecule of Claim 34 wherein the promoter
is a S. lividans, S. coelicolor, S. azuraeus, S. albus, S.
carzinostaticus, S. antifibrinolyticus or S. longisporus
gal operon P2 promoter.

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36. The molecule of Claim 35 wherein the promoter is a S. lividans gal operon P2 promoter.

5

37. The molecule of Claim 34 which further comprises a foreign functional DNA sequence operatively linked to the P2 promoter.

38. A transformed host microorganism or cell comprising the molecule of Claim 37.

10

39. A method of preparing a transformed host microorganism or cell comprising the molecule of Claim 37 which comprises transforming an appropriate host microorganism or cell with such molecule.

40. A recombinant DNA vector comprising the molecule of Claim 37 and, optionally, additionally comprising a replicon.

15

41. A transformed host microorganism or cell comprising the recombinant DNA vector of Claim 40.

42. A method of preparing a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 40 which comprises transforming an appropriate host microorganism with such vector.

20

43. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 40 under suitable conditions such that the functional DNA sequence is expressed.

25

44. A recombinant DNA molecule comprising a Streptomyces gal operon P1 promoter or any regulatable and functional derivative thereof.

30

45. The molecule of Claim 44 wherein the promoter is a S. lividans, S. coelicolor, S. azureus, S. albus, S. carzinostaticus, S. antifibrinolyticus or S. longisporus gal operon P1 promoter.

46. The molecule of Claim 45 wherein the promoter is a S. lividans gal operon P1 promoter.

35

47. The molecule of Claim 44 which further comprises a foreign functional DNA sequence operatively linked to the P1 promoter.

1 48. A transformed host microorganism or cell
comprising the molecule of Claim 47.

5 49. A method of preparing a transformed host
microorganism or cell comprising molecule of Claim 47
which comprises transforming an appropriate host
microorganism or cell with such molecule.

 50. A recombinant DNA vector comprising the
molecule of Claim 47, and, optionally, additionally
comprising a replicon.

10 51. A transformed host microorganism or cell
comprising the recombinant DNA vector of Claim 50.

 52. A method of preparing a transformed host
microorganism or cell comprising the recombinant DNA
vector of claim 50 which comprises transforming an
15 appropriate host microorganism with such vector.

 53. A method of expressing a foreign functional
DNA sequence which comprises cultivating a transformed
host microorganism or cell comprising the recombinant DNA
vector of Claim 50 under suitable conditions such that the
20 functional DNA sequence is expressed.

 54. A method of regulating the expression of a
foreign functional DNA sequence which comprises
cultivating a transformed host microorganism or cell which
contains the recombinant DNA vector of Claim 50 under
25 appropriate conditions such that expression of the
sequence is regulatable.

 55. A recombinant DNA molecule comprising a
Streptomyces gal operon galE gene, or any functional
derivative thereof.

30 56. The molecule of Claim 55 wherein the gene is
a S. lividans, S. coelicolor, S. azuraeus, S. albus, S.
carzinostaticus, S. antifibrinolyticus or S. longisporus
gal operon galE gene.

35 57. The molecule of Claim 56 wherein the gene is
a S. lividans gal operon galE gene.

1 58. The molecule of Claim 55 which further
comprises a foreign functional DNA sequence operatively
linked to the galE gene.

5 59. A transformed host microorganism or cell
comprising the molecule of Claim 58.

60. A method of preparing a transformed host
microorganism or cell comprising the molecule of Claim 58
which comprises transforming an appropriate host
microorganism or cell with such molecule.

10 61. A recombinant DNA molecule comprising a
Streptomyces gal operon galT gene, or any functional
derivative thereof.

15 62. The molecule of Claim 61 wherein the gene is
a S. lividans, S. coelicolor, S. azuraeus or S. albus, S.
carzinostaticus, S. antifibrinolyticus and S. longisporus
gal operon galT gene.

63. The molecule of Claim 62 wherein the gene is
a S. lividans gal operon galT gene.

20 64. The molecule of Claim 61 which further
comprises a foreign functional DNA sequence operatively
linked to the galT gene.

65. A transformed host microorganism or cell
comprising the molecule of Claim 64.

25 66. A method of preparing a transformed host
microorganism or cell comprising the molecule of Claim 64
which comprises transforming an appropriate host
microorganism or cell with such molecule.

30 67. A recombinant DNA molecule comprising a
Streptomyces lividans gal operon galK gene, or any
functional derivative thereof.

35 68. The molecule of Claim 67 wherein the gene is
a S. lividans, S. coelicolor, S. azuraeus, S. albus, S.
carzinostaticus, S. antifibrinolyticus or S. longisporus
gal operon galK gene.

69. The molecule of Claim 68 wherein is a S.
lividans gal operon galK gene.

1 70. The molecule of Claim 67 which further
comprises a foreign functional DNA sequence operatively
linked to the galK gene.

5 71. A transformed host microorganism or cell
comprising the molecule of Claim 70.

72. A method of preparing a transformed host
microorganism or cell comprising the molecule of Claim 70
which comprises transforming an appropriate host
microorganism or cell with such molecule.

10 73. A method of enabling a nongalactose
utilizing host microorganism or cell to utilize galactose
which comprises transforming such host with a recombinant
DNA vector or molecule comprising a Streptomyces gal
operon, or any portion of the Streptomyces gal operon
15 which is adequate to enable such transformed host to
utilize galactose, or any functional derivative thereof.

74. A transformed host prepared by the method of
Claim 73.

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Claims for the Contracting States : AT, ES, GR

1. A method of preparing a transformed host microorganism or cell comprising the molecule which has 5 the following sequence :

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10      -120      -110      -100      -90      -80      -70
      .         .         .         .         .         .
CTA CGC CTC CGC GTT CAG TAA TTC AAC ACT TTT GGT GAT GAA CTT TGT TTC ATT CTC

      -60      -50      -40      -30      -20
      .         .         .         .         .
ATC TGA CAG GGC CGT GGT GGC TTG TGA TGT GTT ATG TTT GAT TGT GTT GGA TGA TTG
                                     galP1

15      -10      1      10      20      30      40
      .         .         .         .         .         .
ACG GGC GTC CTG GTG ACT CAT GGC TGG GTG CAG AGG AGT GCG GCA GTG AAG AAG ACC
      Met Thr His Gly Trp Val Gln Arg Ser Ala Ala Val Lys Lys Thr
      .         .         .         .         .         .
      50      60      70      80      90      100
      .         .         .         .         .         .
TCG ACC CGG CTG GCC GAC GGC CGT GAG CTG GTC TAC TAC GAC CTG CGC GAC GAC ACC
20      Ser Thr Arg Leu Ala Asp Gly Arg Glu Leu Val Tyr Tyr Asp Leu Arg Asp Asp Thr

      110      120      130      140      150
      .         .         .         .         .
GTG CGC GAC GCC GTG GAC CGC CGT CCG CTG GAG CGC ACC GTC ACC ACC TCC GAG GTC
Val Arg Asp Ala Val Asp Arg Arg Pro Leu Glu Arg Thr Val Thr Thr Ser Glu Val

160      170      180      190      200      210
      .         .         .         .         .         .
CGA CGC GAC CCG CTG CTC GGC GAC TCC GCG CCG TCC CGC CTC GCA CCG GCA GGG GCG
25      Arg Arg Asp Pro Leu Leu Gly Asp Ser Ala Pro Ser Arg Leu Ala Pro Ala Gly Ala

      220      230      240      250      260      270
      .         .         .         .         .         .
CAC CTA CCA TCC GCC GGC CGA CCA GTG CCC GCT GTG CCG GTC GGA CGG GGA ACG GCT
His Leu Pro Ser Ala Gly Arg Pro Val Pro Ala Val Pro Val Gly Arg Gly Thr Ala

30      280      290      300      310      320      330
      .         .         .         .         .         .
GAG CGA GAT CCG GCC TAT GAC GTG GTG GTC TTC GAG AAT CCG TTT CCC TCG CTG GCC
Glu Arg Asp Pro Ala Tyr Asp Val Val Val Phe Glu Asn Arg Phe Pro Ser Leu Ala

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		340		350		360		370		380		
5		CCT GAC TCC GCG CGC TGC GAG GTC GTC TGC TTC ACC TCC GAC CAC GAC GCC TCC TTC		Gly Asp Ser Gly Arg Cys Glu Val Val Cys Phe Thr Ser Asp His Asp Ala Ser Phe								
		390		400		410		420		430		440
		GCC GAC CTG ACC GAG GAG CAG GCC CGG CTC GTC GTC GAC GCC TGG ACG GAC CGC ACC		Ala Asp Leu Ser Glu Glu Gln Ala Arg Leu Val Val Asp Ala Trp Thr Asp Arg Thr								
10		450		460		470		480		490		500
		TCC GAG CTG TCC CAT CTG CCC TCC GTT GAA CAG GTC TTC TGC TTC GAG AAC CGG GGC		Ser Glu Leu Ser His Leu Pro Ser Val Glu Gln Val Phe Cys Phe Glu Asn Arg Gly								
		510		520		530		540		550		
15		GCC GAG ATC GGG GTG ACG CTG GGT CAC CCG CAC GGG CAG ATC TAC GCC TAC CCG TTC		Ala Glu Ile Gly Val Thr Leu Gly His Pro His Gly Gln Ile Tyr Ala Tyr Pro Phe								
		560		570		580		590		600		610
		ACC ACC CCC CGC ACC GCC CTG ATG CTC CGT TCA CTC GCC GCC CAC AAG GAC GCG ACC		Thr Thr Pro Arg Thr Ala Leu Met Leu Arg Ser Leu Ala Ala His Lys Asp Ala Thr								
20		620		630		640		650		660		670
		GCG GCG GCG AAC CTG TTC GAC TCC GTG CTG GAG GAG GAG CTG GCC GGT GAG CGG GTC		Gly Gly Gly Asn Leu Phe Asp Ser Val Leu Glu Glu Glu Leu Ala Gly Glu Arg Val								
		680		690		700		710		720		
25		GTC CTC GAG GGT GAG CAC TGG GCC GCC TTC GTC GCG TAC GGC GCG CAC TGG CCG TAC		Val Leu Glu Gly Glu His Trp Ala Ala Phe Val Ala Tyr Gly Ala His Trp Pro Tyr								
		730		740		750		760		770		780
		GAG GTC CAC CTC TAC CCG AAG CGG CGG GTG CCC GAT CTG CTC GGG CTC GAC GAG GCG		Glu Val His Leu Tyr Pro Lys Arg Arg Val Pro Asp Leu Leu Gly Leu Asp Glu Ala								
30		790		800		810		820		830		840
		GCT CGC ACA GAA TTC CCC AAG GTC TAC CTG GAG CTC CTG AGG CGT TTC GAC CGG ATC		Ala Arg Thr Glu Phe Pro Lys Val Tyr Leu Glu Leu Leu Arg Arg Phe Asp Arg Ile								

35

1

5 850 860 870 880 890 900
TTC GGC GAG GGC GAG CCC CCG ACC CCC TAC ATC GCG GCC TGG CAC CAG GCG CCG TTC
Phe Gly Glu Gly Glu Pro Pro Thr Pro Tyr Ile Ala Ala Trp His Gln Ala Pro Phe
 910 920 930 940 950
GGC CAG CTC GAG TTC GAG GGT GTG ACC CCG GAC GAC TTC GCG CTC CAC CTG GAA CTT
Gly Gln Leu Glu Phe Glu Gly Val Thr Arg Asp Asp Phe Ala Leu His Leu Glu Leu
10 960 970 980 990 1000 1010
TTC ACT TCC GCC GTA CGT CCG GCA AGC TGA AGT TCC TCG CCG GCT CCG AAT CCG GCA
Phe Thr Ser Ala Val Arg Pro Ala Ser --- galP2
 1020 1030 1040 1050 1060 1070
15 TGAACC TGTCATCAA CGACGTACCC CCGGAGCGCG CGGCCGAGCG ACTGCGAGAG GTACCGAG
 1080 1090 1100 1110 1120 1130
TTC ATG AGC GGC AAG TAC CTG GTG ACA GGT GGT GCC GGA TAC GTC GGC AGC GTC GTC
Met Ser Gly Lys Tyr Leu Val Thr Gly Gly Ala Gly Tyr Val Gly Ser Val Val
20 1140 1150 1160 1170 1180 1190
GCC CAG CAC TTG GTG GAG GCG GGG AAC GAG GTC GTG CTG CAC AAT CTG TCG ACC
Ala Gln His Leu Val Glu Ala Gly Asn Glu Val Val Val Leu His Asn Leu Ser Thr
 1200 1210 1220 1230 1240
25 GGC TTC CGT GAG GTG TGC CCG CCG GTG CCT CGT TCG TCG AGG CGA CAT CCG GGA CCG
Gly Phe Arg Glu Val Cys Arg Arg Val Pro Arg Ser Ser Arg Arg His Pro Gly Arg
 1250 1260 1270 1280 1290 1300
CGC CAA GTG CGT GGA CCG CTC TCG TTC GAC GGC GTG CTG CAC TTC GCC GCC TTC TCC
Arg Gln Val Arg Gly Arg Leu Ser Phe Asp Gly Val Leu His Phe Ala Ala Phe Ser
30 1310 1320 1330 1340 1350 1360
CAG GTC GGC GAG TCG GTC GTG AAG CCC GAG AAG TAC TCG GAC AAC AAC GTC GGT GCC
Gln Val Gly Glu Ser Val Val Lys Pro Glu Lys Tyr Trp Asp Asn Asn Val Gly Gly
35

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5 1370 1380 1390 1400 1410 1420
ACC ATG GCG CTG CTG GAG GCC ATG CCG GCC GCG GGT GTG CCG CCG CTC GTC TTC TCC
Thr Met Ala Leu Leu Glu Ala Met Arg Gly Ala Gly Val Arg Arg Leu Val Phe Ser

 1430 1440 1450 1460 1470
TCC ACC GCC GCC ACC TAC GCG GAG CCC GAG CAG GTT CCC ATC GTC GAG TCC GCG CCG
Ser Thr Ala Ala Thr Tyr Gly Glu Pro Glu Gln Val Pro Ile Val Glu Ser Ala Pro

10 1480 1490 1500 1510 1520 1530
ACC AGG CCC ACC AAT CCG TAC GCG GCC TCG AAG CTC GCG GTC GAC CAC ATG ATC ACC
Thr Arg Pro Thr Asn Pro Tyr Gly Ala Ser Lys Leu Ala Val Asp His Met Ile Thr

 1540 1550 1560 1570 1580 1590
GGC GAG GCG CCG GCC CAC GGG CTG GCG GCG GTC TCC GTG CCG TAC TTC AAC GTC CCG
Gly Glu Ala Ala Ala His Gly Leu Gly Ala Val Ser Val Pro Tyr Phe Asn Val Ala

 1600 1610 1620 1630 1640
GGC GCG TAC GCG GAG TAC GCG GAG CCG CAC GAG CCC GAG TCG CAT CTG ATT CCG CTG
Gly Ala Tyr Gly Glu Tyr Gly Glu Arg His Asp Pro Glu Ser His Leu Ile Pro Leu

20 1650 1660 1670 1680 1690 1700
GTC CTT CAA GTG GCG CAG GCG AGG CCG GAG GCC ATC TCC GTC TAC GCG GAC GAC TAC
Val Leu Gln Val Ala Gln Gly Arg Arg Glu Ala Ile Ser Val Tyr Gly Asp Asp Tyr

 1710 1720 1730 1740 1750 1760
CCG ACC CCG GAC CGA CCT GTG TGC GCG ACT ACA TCC ACC TCG CCG ACC TGG CCG AGG
Pro Thr Pro Asp Arg Pro Val Cys Ala Thr Thr Ser Thr Ser Pro Thr Trp Pro Arg

25 1770 1780 1790 1800 1810
CCC ACC TGC TGG CCG TGC GCC GCC GCC CCG GCG GAG CAC CTC ATC TGC AAC CTG GCG
Pro Thr Cys Trp Pro Cys Ala Ala Ala Pro Gly Glu His Leu Ile Cys Asn Leu Gly

30 1820 1830 1840 1850 1860 1870
AAC GCG AAC GCG TTC TCC GTC CCG GAG GTC GTC GAG ACC GTG CCG CCG GTG ACC GCG
Asn Gly Asn Gly Phe Ser Val Arg Glu Val Val Glu Thr Val Arg Arg Val Thr Gly

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5 2390 2400 2410 2420 2430 2440
 CGC GAG GCC GGA CAC GAG CTG ACC GCC GCC GAC CTC CAC CTG GCC TCG ACC GTC CCG
 Arg Glu Ala Gly His Glu Leu Thr Gly Ala Asp Val His Leu Ala Ser Thr Val Pro

10 2450 2460 2470 2480 2490
 TCC GCG GCG GCG CTC TCC TCC TCC GCG GCC CTG GAG GTC CGT CCC CTG GCG ATG AAC
 Ser Gly Ala Gly Leu Ser Ser Ser Ala Ala Leu Glu Val Arg Pro Leu Ala Met Asn

15 2500 2510 2520 2530 2540 2550
 GAC CTC TAC GCC CTC GCG CTG CCG GCG TGG CAG CTG GCC CCG CTG TGC CAG CCG GCG
 Asp Leu Tyr Ala Leu Ala Leu Arg Gly Trp Gln Leu Ala Arg Leu Cys Gln Arg Ala

20 2560 2570 2580 2590 2600 2610
 GAG AAC GTC TAC GTC GCG GCC CCC GTC GCG ATC ATC GAC CAG ACC GCG TCC GCG TGC
 Glu Asn Val Tyr Val Gly Ala Pro Val Gly Ile Met Asp Gln Thr Ala Ser Ala Cys

25 2620 2630 2640 2650 2660 2670
 TGC GAG GCG GCG ACG CCC TCT TCC TCG ACA CCC GCG ACC TCT CCC ACC GCG AGA TCC
 Cys Glu Ala Gly Thr Pro Ser Ser Ser Thr Pro Ala Thr Ser Pro Ser Gly Arg Ser

30 2680 2690 2700 2710 2720
 CCT TCG ACC TCG CCG CCG AGG GGA TGC GCC TGC TGG TCG TCG ACA CCC GCG TCA ACC
 Pro Ser Thr Ser Pro Pro Arg Gly Cys Ala Cys Trp Ser Ser Thr Pro Gly Ser Ser

35 2730 2740 2750 2760 2770 2780
 ACT CCC ACA GCG AGG CCG AGT ACG GCA AGC GCC GCG CCG GCT GCG AGA AGG GCG CCG
 Thr Pro Thr Ala Arg Ala Ser Thr Ala Ser Ala Ala Arg Ala Ala Arg Arg Ala Pro

 2790 2800 2810 2820 2830 2840
 CGC TGC TCG GCG TCG ACC CCG TGC GAC GTG CCG TAC GCC GAC CTG GAC GCG GCG CTG
 Arg Cys Trp Ala Ser Thr Arg Cys Asp Val Pro Tyr Ala Asp Leu Asp Ala Ala Leu

 2850 2860 2870 2880 2890
 GAG CCG CTG GCG GAC GAG GAG GAG GTG CCG CCG CTG GTC CCG CAC GTG GTG ACC GAG
 Glu Arg Leu Gly Asp Glu Glu Glu Val Arg Arg Leu Val Arg His Val Val Thr Glu

5

2900			2910			2920			2930			2940			2950			
GAC	GAG	CGC	GTC	GAA	CGG	GTC	GTC	CGC	CTG	CTC	GAG	TCG	CCG	ACA	CCC	GGC	GCA	TCG
Asp	Glu	Arg	Val	Glu	Arg	Val	Val	Ala	Leu	Leu	Glu	Ser	Ala	Thr	Pro	Gly	Ala	Ser

2960		2970		2980		2990		3000		3010								
CGC	CGC	TCC	TCC	TCC	AGG	GCC	ACG	CCT	GCT	GGC	CGA	CGA	CTT	CCG	CAT	CTC	CTG	CCC
Ala	Pro	Ser	Trp	Ser	Arg	Ala	Thr	Pro	Ala	Ala	Arg	Arg	Leu	Pro	His	Leu	Leu	Pro

3020			3030			3040			3050			3060						
CGA	GCT	GGA	CCT	CGT	CCT	CGA	CAC	GGC	CCT	GGC	CTC	GGC	GGC	CCT	CGG	CGC	CGG	ATG
Arg	Ala	Gly	Pro	Gly	Arg	Arg	His	Gly	Pro	Gly	Leu	Arg	Gly	Pro	Arg	Arg	<u>Arg</u>	<u>Met</u>

3070 3080 3090 3100 3110 3120

ACC GGC GGC GGC TTC GGC GGC TCG GCG ATC GTC CTG GTG CAG GCC GCC CGC GTG GAC
Thr Gly Gly Gly Phe Gly Gly Ser Ala Ile Val Leu Val Glu Ala Ala Ala Val Asp

3130 3140 3150 3160 3170 3180
 GCC GTC ACC AAG GCG GTC GAG GAC GCC TTC GCC GCG GCG GCC CTC AAG CGT CCG CGG
 Ala Val Thr Lys Ala Val Glu Asp Ala Phe Ala Ala Ala Gly Leu Lys Arg Pro Arg

3190 3200 3210 3220 3230 3240
 GTC TTC GAG GCG GTG CCT CGG CGG GGC GCG GCG CCT GGT CTG ACG GTC AGC CGA GCC
 Val Phe Glu Ala Val Pro Arg Arg Gly Ala Ala Pro Gly Leu Thr Val Ser Arg Ala
 3250 3260 3270 3280 3290
 GCT TCA CCA GCG TGT ACT CCG TGA TCC CCG GCG GGT AGT CCG GGA TCA CCG ACA TGA
 Ala Ser Pro Ala Cys Thr Pro ---

3300
*
GCT GCT AGC CGC

which comprises transforming an appropriate host micro-organism or cell with such molecule.

2. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA vector comprising the sequence of Claim 1 which comprises transforming an appropriate host microorganism or cell
5 with such vector.

3. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising the recombinant DNA vector of Claim 2 under suitable conditions such
10 that the functional DNA sequence is expressed.

4. A method of regulating the expression of a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell which contains the recombinant DNA vector of Claim 2 under
15 appropriate conditions such that expression of the sequence is regulatable.

5. A method of preparing a transformed host microorganism comprising a recombinant DNA molecule comprising a Streptomyces gal operon P2 promoter
20 expression unit or any functional derivative thereof and a foreign functional DNA sequence operatively linked to such expression unit, which comprises transforming an appropriate host microorganism or cell with such molecule.

25 6. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 5 and, optionally, additionally comprising a replicon which comprises transforming an appropriate host microorganism with
30 such vector.

7. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 5 and, optionally,
35 additionally comprising a replicon, under suitable con-

ditions such that the functional DNA sequence is expressed.

8. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a Streptomyces gal operon P1 promoter regulated region or any regulatable and functional derivative thereof and a foreign functional DNA sequence operatively linked to such regulated region, which comprises transforming an appropriate host micro-
10 organism or cell with such molecule.

9. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 8 and, optionally, additionally comprising a replicon which comprises
15 transforming an appropriate host microorganism or cell with such vector.

10. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising the recombinant
20 DNA vector of Claim 8 and, optionally, additionally comprising a replicon under suitable conditions such that the functional DNA sequence is expressed.

11. A method of regulating the expression of a foreign functional DNA sequence which comprises culti-
25 vating a transformed host microorganism or cell which contains a recombinant DNA vector comprising the molecule of Claim 8 and, optionally, additionally comprising a replicon, under appropriate conditions such that expression of the sequence is regulatable.

12. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a Streptomyces gal operon P2 promoter or any functional derivative thereof and a foreign functional DNA sequence operatively linked to the P2
30

promoter, which comprises transforming an appropriate host microorganism or cell with such molecule.

13. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 12 and, optionally, additionally comprising a replicon, which comprises transforming an appropriate host microorganism with such vector.

14. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising a recombinant DNA molecule of Claim 12 and, optionally, additionally comprising a replicon, under suitable conditions such that the functional DNA sequence is expressed.

15. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a Streptomyces gal operon P1 promoter or any regulatable and functional DNA sequence operatively linked to the P1 promoter, which comprises transforming an appropriate host microorganism or cell with such molecule.

16. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 15 and, optionally, additionally comprising a replicon, which comprises transforming an appropriate host microorganism with such vector.

17. A method of expressing a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell comprising a recombinant DNA vector comprising the molecule of Claim 15 and, optionally, additionally comprising a replicon, under suitable conditions such that the functional DNA sequence is expressed.

18. A method of regulating the expression of a foreign functional DNA sequence which comprises cultivating a transformed host microorganism or cell which contains a recombinant DNA vector comprising the molecule of Claim 15 and, optionally, additionally comprising a replicon, under appropriate conditions such that expression of the sequence is regulatable.

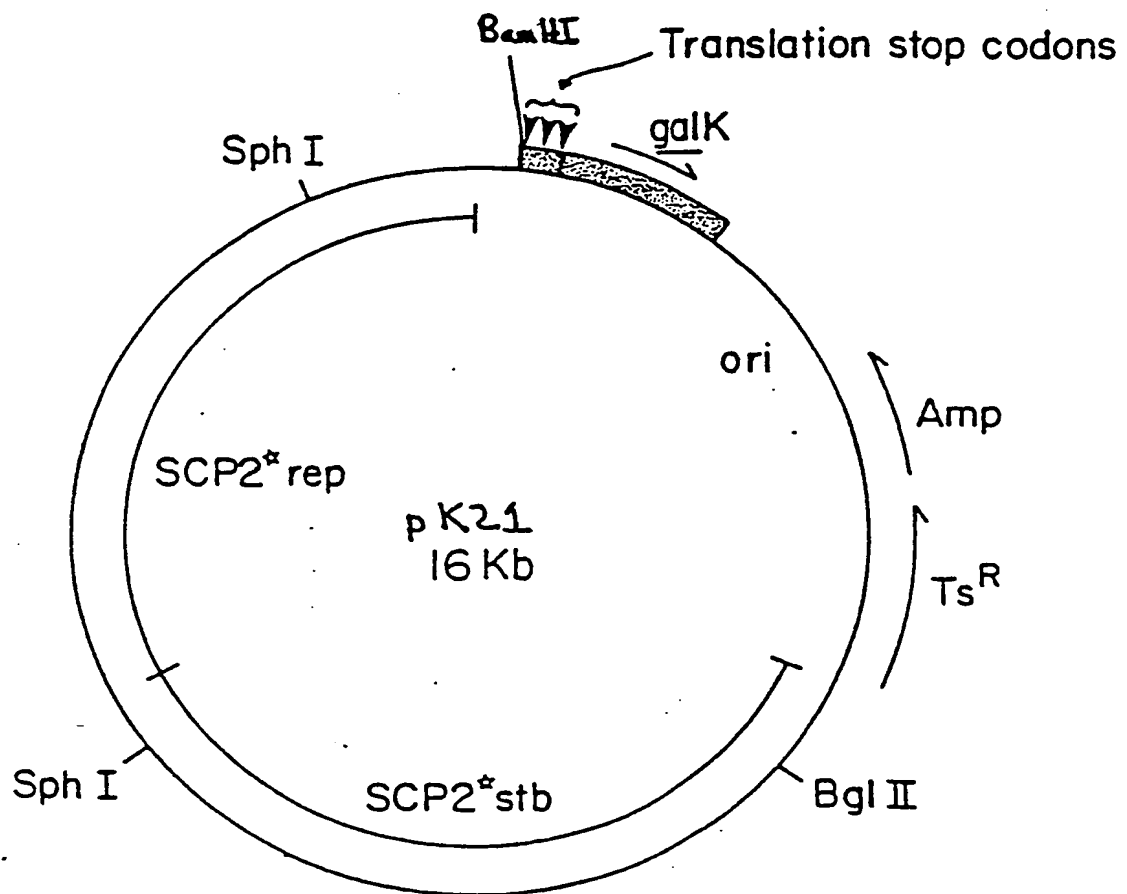
19. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a Streptomyces gal operon galE gene or any functional derivative thereof and a foreign functional DNA sequence operatively linked to the galE gene, which comprises transforming an appropriate host microorganism or cell with such molecule.

20. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a Streptomyces gal operon galT gene or any functional derivative thereof and a foreign functional DNA sequence operatively linked to the galT gene, which comprises transforming an appropriate host microorganism or cell with such molecule.

21. A method of preparing a transformed host microorganism or cell comprising a recombinant DNA molecule comprising a Streptomyces lividans gal operon galK gene or any functional derivative thereof and a foreign functional DNA sequence operatively linked to the galK gene, which comprises transforming an appropriate host microorganism or cell with such molecule.

22. A method of enabling a nongalactose utilizing host microorganism or cell to utilize galactose which comprises transforming such host with a recombinant DNA vector or molecule comprising Streptomyces gal operon, or any portion of the Streptomyces gal operon which is adequate to enable such transformed host to utilize galactose, or any functional derivative thereof.

Figur 1



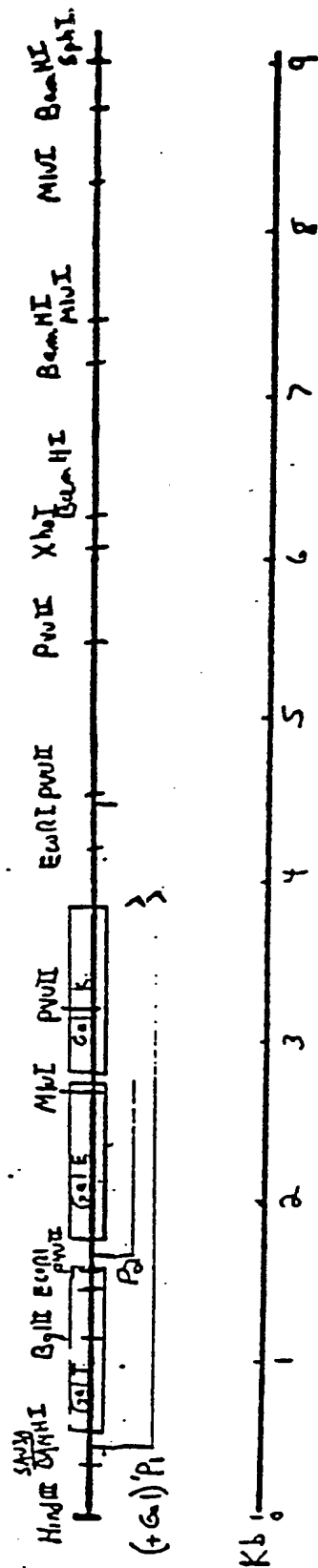
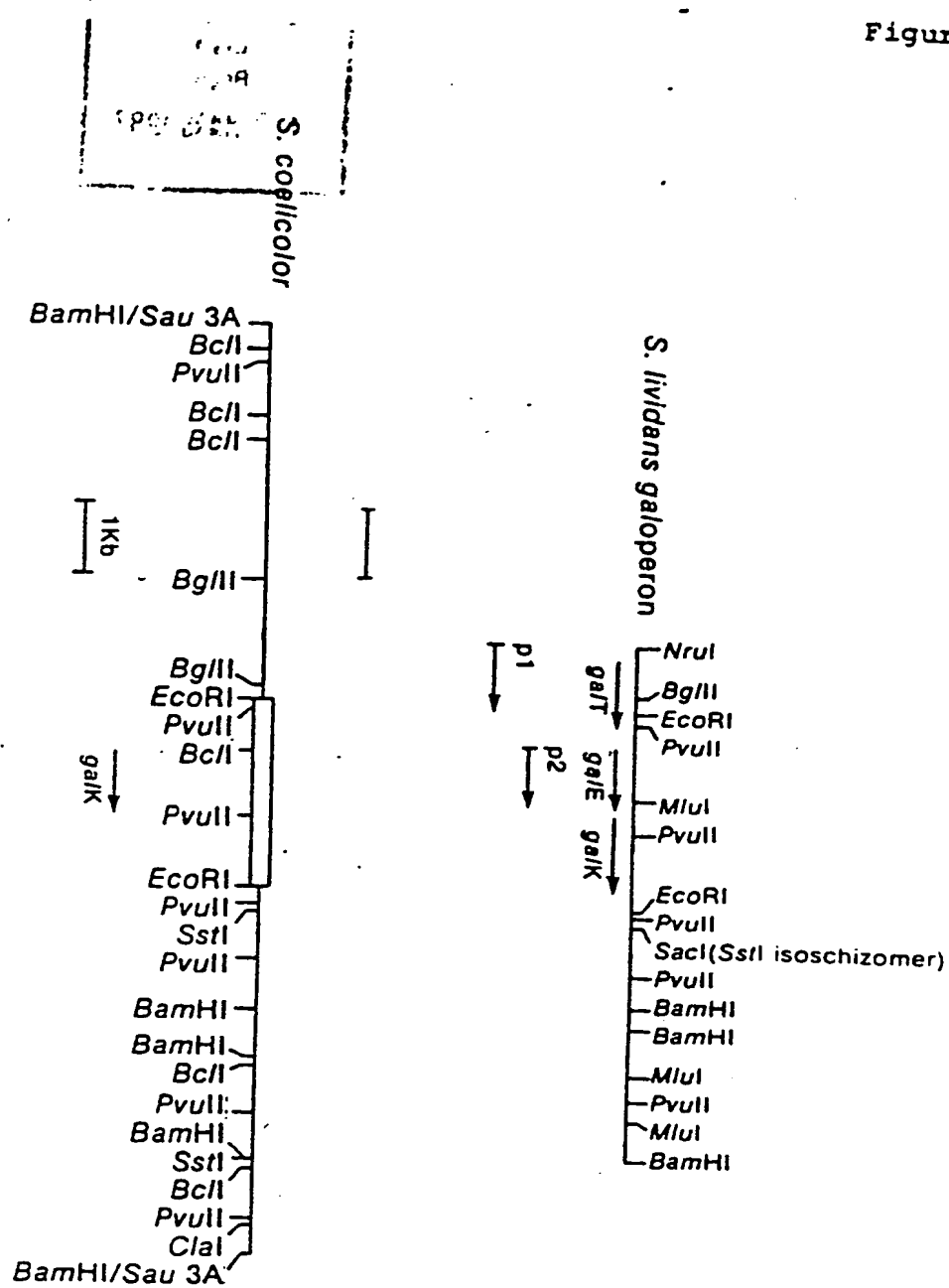


Figure 2

Figure 3



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EUROPEAN PATENT APPLICATION

②① Application number: 87870026.9

⑤① Int. Cl.³: **C 12 N 15/00**
C 12 -N 1/20

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30.01.87 US 9419

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AT BE CH DE ES FR GB GR IT LI LU NL SE

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⑦④ Representative: Tasset, Gérard
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⑤④ The gal operon of streptomyces.

⑤⑦ A recombinant DNA molecule comprising the *Streptomyces gal* operon *galK* gene; *galE* gene; *galT* gene; P1 promoter; P2 promoter; P2 promoter expression unit; P1 promoter regulated region, or the entire *Streptomyces gal* operon is prepared.

EP 0 235 112 A3



European Patent
Office

EUROPEAN SEARCH REPORT

0235112

Application Number

EP 87 87 0026

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
D,A	GENE, vol. 40, no. 2/3, 1985, pages 191-201, Elsevier Science Publishers, Amsterdam, NL; M.E. BRAUNER et al.: "Characterization of Streptomyces promoter sequences using the Escherichia coli galactokinase gene" ---		C 12 N 15/00 C 12 N 1/20
D,A	NUCLEIC ACIDS RESEARCH, vol. 13, no. 6, 1985, pages 1841-1853, IRL Press Ltd, Oxford, GB; C. DEBOUCK et al.: "Structure of the galactokinase gene of Escherichia coli, the last (?) gene of the gal operon" ---		
A	EP-A-0 187 630 (SMITHKLINE BECKMAN CORP.) ---		
A	ABSTRACTS OF THE ANNUAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, vol. 84, no. 0, 1984, abstract no. H98; W. BURNETT et al.: "Transcriptional and translational regulatory elements in the streptomyces - lividans beta-gal operon" -----		
			TECHNICAL FIELDS SEARCHED (Int. Cl. 4)
			C 12 N
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 02-08-1988	Examiner PULAZZINI A.F.R.
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ----- & : member of the same patent family, corresponding document			